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ORIGINAL ARTICLES

CHAULMOOGRA OIL IN THE TREATMENT OF TUBERCULOSIS*

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THE use of chaulmoogra oil in the treatment of leprosy and various cutaneous diseases has been recorded since 1596 A.D. The oil is obtained from the seeds of Taraktogenos Kurzii, King Family, Flacourtiaceæ, a tree native to the forests of Burma and eastern India. The tree produces a large fruit with numerous seeds imbedded in the pulp and it is from these seeds that the oil is expressed. The term "Chaulmoogra odorata" was applied to the tree by Roxburgh in 1814. Siam produced a tree closely related to the gynocardia, the seeds of which were used by the ancient Chinese for medicinal purposes.

The crude oil, expressed cold from the seeds, is a solid, possessing light brown color, pungent odor, highly disagreeable and nauseating taste. The early method of administration was by external application in conjunction with other oils. This was superseded by an oral administration. The nauseating and irritating properties have been an obstacle to its use in modern medicine. The problem presented was that of retaining the active principle of the oil and at the same time eliminating the undesirable effects. Little progress was made until 1890, when hope of a possible cure for leprosy aroused by the heroic efforts of the Belgian priest, Father Damien, stimulated further investigation with chaulmoogra oil.

The oral administration of the drug was unsatisfactory because of the unavoidable gastric disturbance which accompanied it. Investigators accordingly resorted to subcutaneous and intramuscular injection. Encouraging results were obtained by mixing the oil with camphor and resorcin which reduced its irritating properties and rendered it more fluid. In Dyer's opinion the drug was most efficacious when combined with camphor and resorcin.

Hollman and Dean recently isolated the fatty acids of chaulmoogra oil

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separating them into groups by fractional crystallization. They converted the individual groups or fractions into each respective ethyl ester in which form they used the drug in treating leprosy. Marked improvement was reported in cases treated over a period of four months, with these fractions.

Sir Leonard Rogers was the first to employ the soluble sodium and potassium salts of chaulmoogra oil. His investigations conducted in 1915-16-17 using the salts orally and intravenously gave exceedingly promising results, a cure being effected in some cases and distinct improvement in all patients treated.

Walker and Sweeney reported their findings in an investigation in March, 1920. They determined that the action of chaulmoogra oil is bactericidal rather than physiologic, the active principle is the fatty acids; the action is specific for acid-fast bacteria and is a function of the carbon ring structure of the molecule of chaulmoogric acids found only in chaulmoogra oil and oils of trees closely related to the gynocardia odorata. They suggested that it may be of benefit in the treatment of tuberculosis and are conducting investigations to determine this point.

Numerous investigators have apparently come to the same conclusion with reference to the treatment of leprosy with chaulmoogra oil or its active principles, i. e., a high percentage of patients are improved and a few cures are recorded. The similarity in morphology and staining characteristics of leprosy bacilli and tubercle bacilli and the specific action of chaulmoogra and its related oils on the acid-fast group of bacteria, furnish a scientific basis for using them in the treatment of tuberculosis. With the following objects in view our work was started early in January, 1920. The acid fractions of chaulmoogra oil obtained by the method of Hollman and Dean and modified by T. B. Aldrich were used in the first attempt. T. B. Aldrich of this institution prepared all the fractions used throughout the investigation.

The scope of our efforts is as follows:

1. To determine the most active, soluble and least irritating fraction obtained from the oil.

2. To determine the most effective method of administration.

3. To determine what pathological effect, if any, is induced by large doses of the fractions of the oil.

4. To determine the bactericidal properties for tubercle bacilli *in vitro*.

5. To determine whether its use will inhibit or arrest the development of artificially induced tuberculosis in guinea pigs.

Fifty healthy guinea pigs, male and female, averaging about 300 grams were selected. In about two weeks the experiment was terminated when an epidemic of bronchisepticus and pneumonia appeared in our animal house. So many pigs were lost from these two diseases that it was necessary to select another group of fifty and repeat the experiment. The only definite and positive information gathered from our first group of fifty pigs, was, that the ethyl esters were exceedingly irritating when administered intraperitoneally and slightly toxic in 1 c.c. doses; that the peritoneum showed chronic inflammation, particularly at the point of injection. The spleen and liver were hypertrophied and showed beginning fatty degeneration; that the fatty acids were

very insoluble; that a virulent suspension of the tubercle bacilli incubated 48 hours at 37° C. with each of the acid fractions in a dilution of 1:10,000 failed to kill any of the twelve pigs infected or to grow when transplanted.

The second group of fifty pigs were selected February, 1920, and the work started as before. We used the acid sodium salts of chaulmoogra oil to replace the ethyl esters. Nine days from the date the investigation was started a second epidemic was encountered and so many pigs were lost that it was necessary to discard the remaining and select another group of fifty. The information gathered from this attempt, was that the acid sodium salts of the four fractions, A, B, C, and D, of chaulmoogra acid are fairly soluble and far less irritating when administered intraperitoneally. They were not toxic in 1 c.c. doses.

A third group of forty-eight pigs was begun on July 27 under most favorable conditions. We used a large concrete building 20x40x16 ft., with 14 ordinary sized windows and one door. This room was equipped with one dozen 4x2 pens which were well ventilated. This place was made to assume, as near as possible, the proportions, conditions, and surroundings of the loft where the pigs were raised. They were given an abundance of the same foods that they had always received. The pens were kept clean and the water receptacle full of fresh water at all times. The pigs were kept free from all body vermin. The soluble acid sodium salts of the four acid fractions respectively were prepared. We used a 1 per cent solution of each of the salts of the fractions prepared according to the methods of Hollman and Dean, Walker and Sweeney and modified by Aldrich.

On July 27 the twenty-four pigs were inoculated with 0.5 c.c. of a heavy suspension of virulent tubercle bacilli isolated by the writer from a patient suffering with pulmonary tuberculosis. Twelve of these pigs were selected and designated as Group I or known as our tuberculous controls. These pigs were placed in two pens, six in each and not molested further. The remaining twelve of the twenty-four inoculated with tubercle bacilli were designated as Group II. This group was subdivided into four groups of three pigs each and the first subdivision containing three pigs was designated as fraction "A" group. Each of these animals received six 0.2 c.c. doses of the "A" fraction of the acid sodium salt of chaulmoogra oil intraperitoneally, at three day intervals, and nine 0.3 c.c. doses, administered the same way at the same intervals. The second subdivision, containing three pigs was designated as fraction "B" group. Each of these animals received six 0.2 c.c. doses of the "B" at three day intervals, and nine 0.3 c.c. doses administered the same way at the same intervals. The third subdivision containing three pigs was designated as fraction "C" group. Each of these animals received six 0.2 c.c. doses of the "C" at three day intervals, and nine 0.3 c.c. doses administered the same way at the same intervals. The fourth subdivision containing three pigs was designated as fraction "D" group. Each of these animals received six 0.2 c.c. doses of the "D" fraction of the acid sodium salt of chaulmoogra oil, intraperitoneally, at three day intervals, and nine 0.3 c.c. doses administered the same way at the same intervals.

A third group of eight pigs was selected and designated as Group III or our normal controls. They were placed in two pens, four in each and not

molested further except they were weighed every third day. The object in retaining this group of animals was to have a control for the living conditions.

A fourth group of twelve pigs was selected and designated Group IV, being placed in four pens, three in each pen. Each of the three pigs in pen (A) received six 0.2 c.c. doses of the "A" fraction of acid sodium salts of chaulmoogra oil intraperitoneally at three day intervals and nine 0.3 c.c. doses administered the same way at the same intervals. This group of pigs was designated fraction "A" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II, fraction "A" group infected with tubercle bacilli. Each of the three pigs in pen "B" received six 0.2 c.c. doses of "B" fraction of the acid sodium salts of chaulmoogra oil intraperitoneally at three day intervals and nine 0.3 c.c. doses administered the same way at the same intervals. This group of pigs was designated Fraction "B" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II, fraction "B" group infected with tubercle bacilli. Each of the three pigs in pen "C" received six doses 0.2 c.c. of "C" fraction of the acid sodium salt of chaulmoogra oil intraperitoneally at three day intervals. This group of pigs was designated fraction "C" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II fraction "C" group infected with tubercle bacilli. Each of the three pigs in pen "D" received six 0.2 c.c. doses of fraction "D" of the acid sodium salt of chaulmoogra oil intraperitoneally at three day intervals and nine 0.3 c.c. doses administered the same way at the same intervals. This group of pigs was designated fraction "D" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II, fraction "D" group infected with tubercle bacilli.

A fifth group of four pigs was selected and placed in a pen. These pigs were the toxicity and quantity test animals. One pig was selected, tagged and marked "A," another was tagged and marked "B," another "C" and the last "D." Pig "A" received 0.5 c.c. of fraction "A" of the acid sodium salt of chaulmoogra oil intraperitoneally at three day intervals until a total of 4 c.c. was administered. Pigs "B," "C" and "D" were treated the same way, using fraction "B," "C" and "D" of the acid sodium salt of chaulmoogra oil respectively for pigs "B," "C" and "D." These animals were autopsied 24 hours after they had received the injection.

This accounts for the disposition of the original 48 pigs which were used in the investigation.

To make it possible to draw a comparison, from a pathologic standpoint, between our Group I (tuberculous controls) and our Group II (treated pigs infected with tuberculosis) it was necessary to have a constant factor. We chose time as this factor. It was, therefore, necessary for us to kill a pig from Group II (treated pigs infected with tuberculosis) when a pig from Group I (tuberculous controls) died. This system kept the number of days lived by each group uniform and allowed us to study the gross pathology at autopsy, if any, and supplied us with material for microscopic study. Our next step was to devise a scaling system which would also be constant

for both Groups I and II. The following was used for making counts. Before giving the scaling outline the writer wishes to state that he autopsied and scaled the animals first, keeping this as a permanent record, then he removed all marks of identification from the animal and his assistant was allowed to do the scaling. All throughout the investigation there was never more than a difference of one between our counts and often times our totals were the same. We may, therefore, assume that this factor was constant enough to give us grounds for basing an opinion on one phase of the investigation.

PATHOLOGIC SYSTEM OF SCALING

Glandular Involvement
1. Glands enlarged but no nodules visible.
2. Glands enlarged and few nodules present.
3. Glands enlarged and all axillary glands showing nodules or tubercuous growth.

Peritoneal wall, Diaphragm, Mesentery
1. Granular appearance with a few scattered tubercles.
2. Tubercles more thickly distributed, but still discrete.
3. Tubercles coalescing, forming solid greyish furry coat on peritoneum, or heavy cheesy crust.

Spleen
1. Granular or slightly enlarged, no tubercles visible.
2. Enlarged, congested, small tubercles scattered throughout tissue.
3. Enormously enlarged, large coalescing areas, caseous or softening with presence of pus, or cheesy areas.

Pancreas
1. Somewhat enlarged.
2. Enlarged, showing tuberculous nodules and beginning to harden.
3. Very large and hard, nodules very prominent, adhesions to adjacent tissues.

Liver
1. Not enlarged but appearing either slightly granular in consistency or mottled in color.
2. Slightly enlarged with minute discrete tubercles apparent.
3. Enlarged, tubercles coalescing, or caseous areas or soft granular areas or both.

Lungs
1. Slightly granular or faint pearly areas. Appearance decidedly not normal.
2. Tubercles definitely present but small and widely scattered or confined to one lobe or portion of a lobe.
3. All tissue heavily studded with coalescing nodules, congested areas and pus present.

Fluids
1. Slight excess in either or both body cavities.
2. Large excess in either cavity.
3. Excessive fluids or pus in both cavities.

Kidney
1. Capsule or outer membrane of one or both kidneys showing tubercles.
2. Capsule showing many tubercles, surface beneath mottled or corrugated.
3. Capsule showing many tubercles, also upon sectioning tubercles exhibited.

SUMMARY

One per cent solutions of the soluble acid sodium salt of the four acid fractions of chaulmoogra oil were apparently the most active, soluble and least irritating when administered hypodermatically. There was a conspicuous absence of the drug in the peritoneal cavity of an animal when it came to autopsy although less than 24 hours had elapsed after an heroic dose.

Charts "D" and "E" show a marked gain in weight for the animals which received the chaulmoogra salts, probably indicating their complete

GROUP I PIGS (TUBERCULOUS CONTROLS)

PIG IDENTIFICATION	DATE OF INOCULATION	AMOUNT OF VIRULENT HUMAN TUBERCLE USED	WEIGHT OF PIG AT INOCULATION	WEIGHT AT AUTOPSY	DAYS LIVED	PATHOLOGY COUNT SCALED	L = LIVING D = DIED K = KILLED
7—A	July 27-20	0.5 c.c.	440	360	48	21	D
7—B	" " "	"	350	320	28	22	D
7—C	" " "	"	410	295	74	20	D
7—D	" " "	"	510	430	40	16	D
7—E	" " "	"	475	385	40	21	D
7—F	" " "	"	500	395	42	20	D
8—A	" " "	"	375	340	78	20	D
8—B	" " "	"	520	585*	85*		L
8—C	" " "	"	380	220	29	19	D
8—D	" " "	"	395	325	41	21	D
8—E	" " "	"	490	445	48	19	D
8—F	" " "	"	420	495	37	21	D
Average		0.5 c.c.	438.75	382	49	20	

Average loss in weight during the 49 days lived is 56 grams

*Pig is living on this date.

GROUP II PIGS (INFECTED WITH HUMAN TUBERCULOSIS AND TREATED)

PIG IDENTIFICATION	DATE OF INOCULATION	AMOUNT OF VIRULENT HUMAN TUBERCLE USED	WEIGHT OF PIG AT INOCULATION	WEIGHT AT AUTOPSY	DAYS LIVED	PATHOLOGY COUNT SCALED	TOTAL AMOUNT OF 1% ACID SODIUM SALT OF CHAUL. ADMINISTERED	TOTAL NUMBER OF TREATMENTS	L = LIVING D = DIED K = KILLED
1—A	July 27-20	0.5 c.c.	525	625*	85*		3.9 c.c.	15	L
1—B	"	"	455	420	40	15	3 "	12	K
1—C	"	"	375	350	42	15	3.9 "	15	K
2—A	"	"	325	275	40	11	2.4 "	10	K
2—B	"	"	315	270	48	16	3.3 "	13	K
2—C	"	"	560	470	74	13	3.9 "	15	K
3—A	"	"	320	410	78	13	3.9 "	15	K
3—B	"	"	405	355	28	9	1.8 "	8	K
3—C	"	"	415	440	48	15	3.9 "	15	K
4—A	"	"	420	370	29	10	2.1 "	9	K
4—B	"	"	370	285	37	12	2.4 "	10	D
4—C	"	"	480	410	41	17	3.9 "	15	K
Average			413.75	390	49	13.33	3.2	12.6	

Average Loss in weight for 49 days Lived is 23 grams

*Pig is living on this date.

assimilation. Basing our opinion on the findings in Group II we believe fraction "A" and "B" most potent.

No pathologic findings which could be attributed to the drug were found at necropsy of pigs receiving the chaulmoogra salts, as exhibited by Group V pigs. These pigs gained weight during the administration of the drug. A 1 per cent solution of the acid sodium salts of all the acid fractions of chaulmoogra oil we used were found to be nontoxic as shown by the fact

CHART FOR COMPARING GROUP I WITH GROUP II

PIG IDENTIFICATION	GROUP NO.	DAYS LIVED	LOSS OR GAIN IN WEIGHT	PATHOLOGY COUNTS SCALED	NET POINTS CREDITED GR. II IN WEIGHT	NET POINTS CREDITED GR. II IN COUNTS	NET POINTS CREDITED GR. I IN WEIGHT	NET POINTS CREDITED GR. I IN COUNTS	NET POINTS DEBITED GR. II IN WEIGHT	NET POINTS DEBITED GR. II IN COUNTS	NET POINTS DEBITED GR. I IN WEIGHT	NET POINTS DEBITED GR. I IN COUNTS
1—A	II	85*	+100*		35							
8—B	I	85*	+ 65*								35	
1—B	II	40	− 35	15	10	6						
7—E	I	40	− 45	21							10	6
1—C	II	42	− 25	15	80	5						
7—F	I	42	−105	20							80	5
2—A	II	40	− 50	11	30	5						
7—D	I	40	− 80	16							30	5
2—B	II	48	− 45	16		5			10			
7—A	I	48	− 35	21			10					5
2—C	II	74	− 90	13	25	7						
7—C	I	74	−115	20							25	7
3—A	II	78	+ 90	13	125	7						
8—A	I	78	− 35	20							125	7
3—B	II	28	− 50	9		13			20			
7—B	I	28	− 30	22			20					13
3—C	II	48	+ 25	15	70	4						
8—E	I	48	− 45	19							70	4
4—A	II	29	− 50	10	110	9						
8—C	I	29	−160	19							110	9
4—B	II	37	− 85	12		9			160			
8—F	I	37	+ 75	21			160					9
4—C	II	41	− 70	17		4						
8—D	I	41	− 70	21								4
Average		49.1			40	6						

*Pigs living on this date.

that no pigs were lost from groups IV or V; on the other hand all showed a marked increase in weight after the administration of heavy doses. Group IV curve suggests the presence of a vitamine or some element which has a marked influence on the weight of the pigs.

The peritoneal administration in the case of guinea pigs was found to produce no undesirable effects. In this case peritoneal administration was found to be a method by which the salts may be rapidly absorbed by the body.

We found the acid sodium salt of Chaulmoogra oil has a specific bactericidal action on tubercle bacilli. Sweeney and Walker showed it was specific for the acid-fast group in a 1:100,000 dilution. We were only able to prove its bactericidal properties for tubercle bacilli in a dilution of 1:10,000.

Of the 12 pigs inoculated with tuberculosis and not treated, all died except one, of the 12 pigs treated only one died.

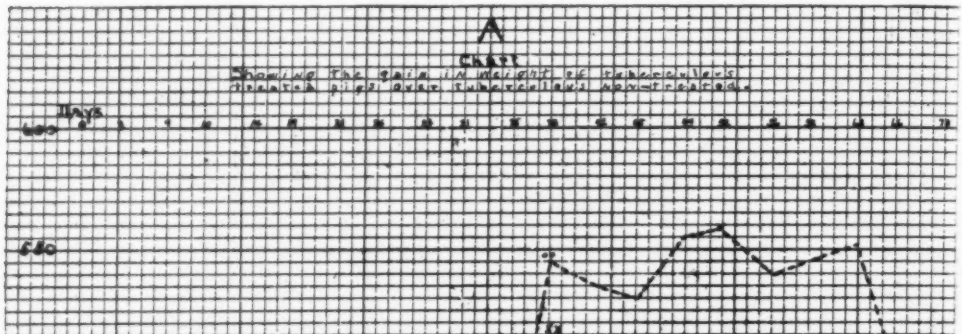
A marked difference in the pathologic findings between pigs which were and were not treated was observed, the advantage being in favor of the treated pigs.

Treated pigs showed an average gain of 49 grams over the ones not treated; the time factor being kept constant.

One pig from Group II which was treated with fraction "A" gave birth to two young ones after the 18th treatment.

More evidence in favor of Group II as compared to I will be found upon close observation and comparison of Charts A, B, and C. It will be noted in Charts A, B and C that at the close of the investigation there is a distinct advantage Group II shows over Group I.

We have more work under way to verify these findings inasmuch as this is a preliminary report.



CONSIDERATION OF CHART A

Chart A is based on the fact that when a pig in Group I died an animal from Group II was killed and the average weight of the remaining animals in each group recorded by the respective curves.

We find the following factors most prominent when Group I pigs (tuberculous controls) are considered.

1. That the average group gain for the first seven days was normal when compared with our normal controls.

2. From the seventh (the point of inoculation) to the seventeenth day the average loss in weight was 43 grams per pig.

3. From the seventeenth to the thirty-eighth day the average gain per pig in the group was 115 grams. This is explained by the fact that the light weight pigs were first to die.

4. From the thirty-eighth to the seventieth day the average loss per pig in the group was 115 grams.

5. From point of inoculation to end of investigation a loss of 43 grams per pig was sustained.

We find the following factors most prominent when Group II pigs (infected with tuberculosis and treated) are considered.

1. The average gain for this group for the first seven days was normal when compared with our normal controls.

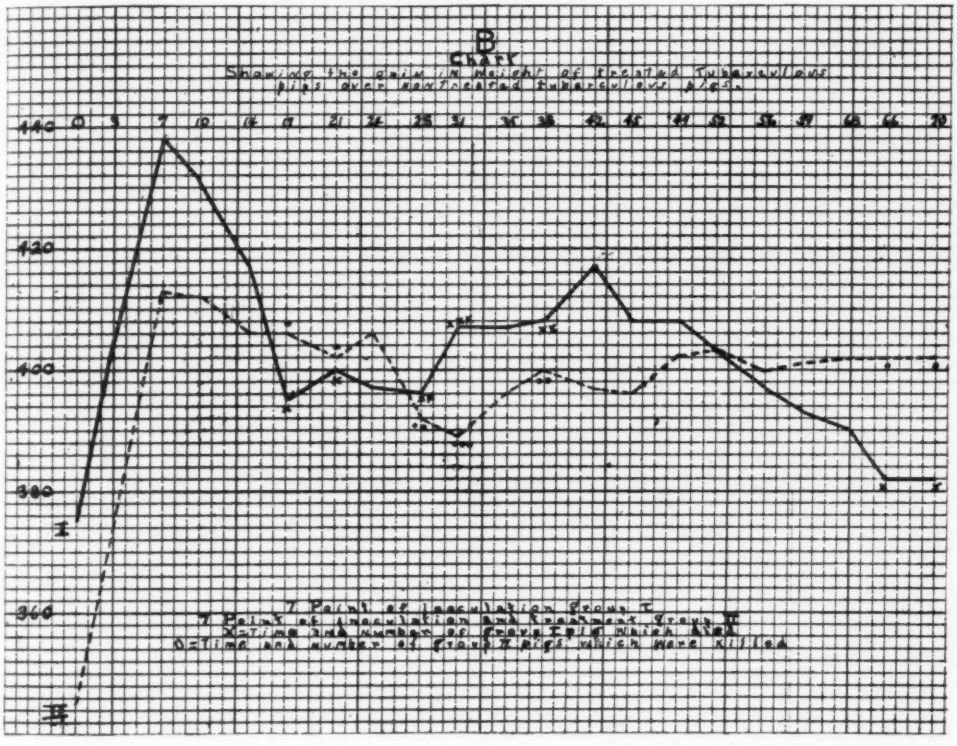
2. From the seventh (point of inoculation and treatment) to the seventeenth day the average loss in weight was two grams, which is not abnormal.

3. From the seventeenth to the thirty-eighth day there was an average gain of 134 grams per pig. This is partially explained by the fact that we tried to select pigs to kill whose weights approximated those of the dead pigs of Group I.

4. From the thirty-eighth to fifty-second day there was an average group gain of 11 grams.

5. From the fifty-second to the seventieth day there was an average loss of 51 grams per pig.

6. From the seventh to seventieth day an average gain of 92 grams per pig.



CONSIDERATION OF CHART B

In drawing Chart B the weight of each pig at necropsy which died or was killed was retained as a constant factor throughout the remainder of the investigation. The points on the chart were the average weights for the entire twelve pigs of each respective group.

The following points on this chart appear most interesting when Group I pigs (tuberculous controls) are considered.

1. The entire group individually and as a unit lost weight uniformly and gradually.

2. The gain in group weight during the first week coincided with the gain in weight of the other normal groups.

3. This group started with each pig averaging 25 grams more than Group II, at point of inoculation.

4. Each animal in the group lost an average of 43 grams the first ten days after inoculation. This is probably due to the virulence of the organism used.

5. All animals in the group died except one.

6. A total loss of an average of 56 grams per pig was sustained from the date of inoculation to the end of the investigation.

7. The pathology count scaled for this group averages 20 per pig.

Consideration of Group II pigs infected with tuberculosis and treated.

1. The entire group individually and as a unit lost weight uniformly and gradually but the amount lost could not be considered abnormal.

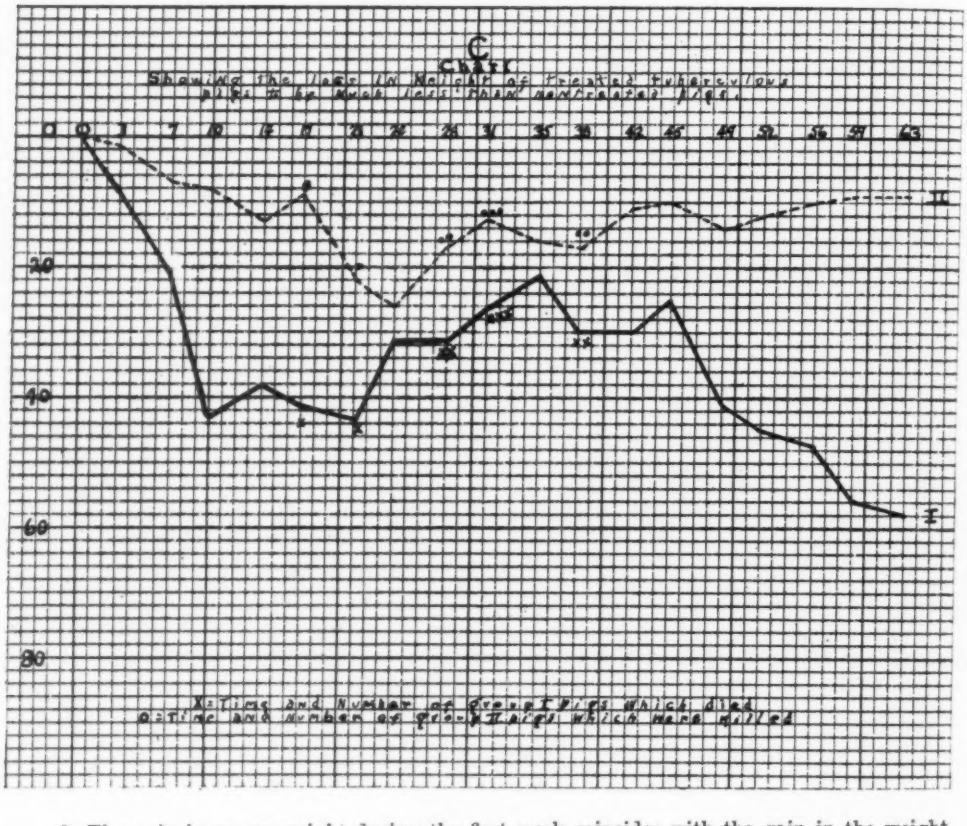


Chart
Showing the loss in Weight of Treated Tuberculous pigs as Much less than Non-Treated pigs.
0 1 7 10 14 17 21 24 28 31 35 38 42 45 49 52 56 59 63
II
I
30
40
60
80
X=Time and Number of Group I Pigs which died
O=Time and Number of Group II pigs which were killed

2. The gain in group weight during the first week coincides with the gain in the weight of the normal group.

3. This group started with each pig averaging 25 grams less than Group I. This group overcomes this handicap throughout the investigation.

4. Each animal in this group lost an average of 7 grams (which is not considered an abnormal fluctuation) the first ten days after inoculation.

5. All animals in the group were killed except one.

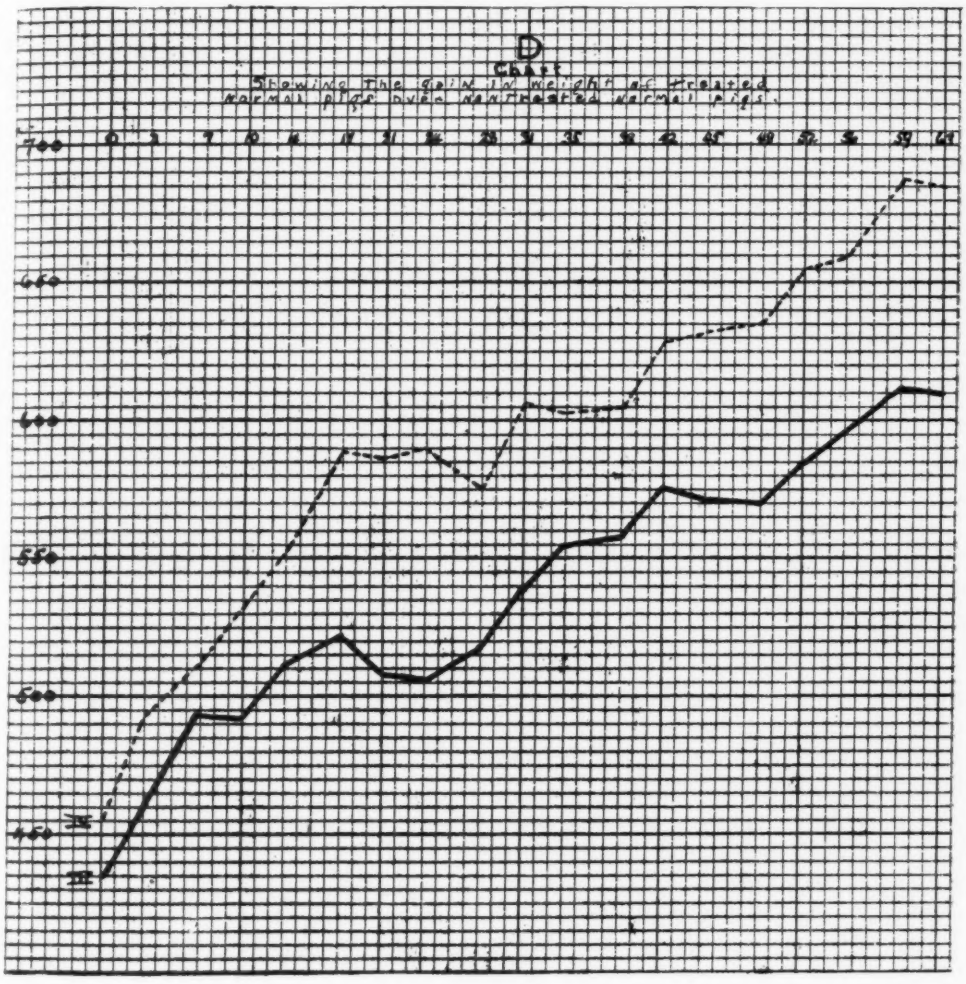
6. The highest point reached by this group was an average of 406 grams.

7. A total loss of an average of 11 grams per pig was noted from the point of inoculation to the end of the investigation.

8. The pathology count scaled for this group averages 13.33 per pig.

Consideration of Chart C

In drawing Chart C the weight at necropsy of the pigs which died or were killed was retained as a constant factor throughout the remainder of the investigation. The points on the chart represent the average loss or gain for the entire twelve pigs based on the original weight of each respective group. Example, if Group I's original weight is 413 grams per pig and at the period of the third weighing is 406 grams this group sustains an average loss of 7 grams per pig over a period of ten days.



Consideration of Group I pigs (tuberculous controls)

1. From point of inoculation, which is charted zero, to the tenth day there was a loss of 43 grams per pig.

2. From the tenth to the thirty-fifth day there was a gain of twenty-two grams per pig.

3. From the thirty-fifth to the sixty-third day there was a loss of thirty-seven grams per pig.

4. From zero to the sixty-third day there was a loss of an average of 58 grams per pig for the group.

5. The entire group lost weight sporadically.

Consideration of Group II Pigs (infected with tuberculosis and treated)

1. From point of inoculation, which is charted zero, to the tenth day, there was a loss of eight grams.
2. From the tenth to the thirty-fifth day there was a loss of eight grams.
3. From the thirty-fifth to the sixty-third day there was a loss of seven grams.
4. From zero to the sixty-third day there was a loss of nine grams.
5. The entire group lost weight uniformly and gradually.

CONSIDERATION OF CHART D

In drawing Chart D the average weights at successive weighing periods of the entire number of pigs of each respective group were recorded as points on the chart, eight pigs being used for Group III and twelve for Group IV.

Consideration of Group III Pigs (Normal controls)

1. None of these pigs died during the course of the investigation.
2. Individually and as a group the pigs showed a steady and gradual increase in weight from the date of inoculation of Groups I and II to the end of the investigation.
3. The entire average gain in weight for the group is 176 grams.
4. This group weighed at start twenty-one grams less than Group IV, and at the end of the investigation 75 grams less than Group IV.
5. The average gain for a three day period was 9.7 grams.

Consideration of Group IV Pigs (Injected with chaulmoogra oil at same periods and in same amounts as pigs of Group II)

1. None of these pigs died during the course of the investigation.
2. All showed a steady and marked increase in weight from the date of first treatment to the end of the investigation.
3. The entire average gain in weight for the group is 230 grams.
4. The group weighed at start 21 grams more than Group III and at the end of the investigation 75 grams more, a gain per pig of 54 grams over the normal pigs.
5. The average gain for a three-day period was 12.7 grams.

The authors wish to thank Dr. E. M. Houghton, at whose suggestion this work on chaulmoogra oil was undertaken and for his kind criticisms and suggestion in carrying it out. Also, we wish to acknowledge the assistance given by Mr. Clark.

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THE PHARMACOLOGIC ACTION OF LEAD IN ORGANIC COMBINATION*

By E. C. Mason, M.D., Cincinnati, Ohio

INTRODUCTION

THE frequent occurrence of lead poisoning, accompanying the extensive use of lead compounds in the various arts and trades, has made the subject of "Lead Poisoning" one of wide-spread interest. This is evidenced by the fact that one of the oldest works on Trade Hygiene (by Stockhausen) is entitled, *"De lithargyrii fumo noxio, morbifico ejusque metallico frequentiori morbo vulgo dicto hüttenkatze,"* Gaslar, 1556.

The deaths occurring from lead poisoning in England and Wales during the ten years 1883 to 1892 were no less than 1043.[1] Of this number three were suicidal; the remaining 1040 were mainly from the manufacture of white lead and from the use of lead in the arts, or from accidental contamination of foods. For the ten years[2] ending 1909, 8,973 cases of lead poisoning with 667 deaths were reported to the Home Office (England) as occurring in 18 industries, but Legge points out that there has been in this period a reduction of more than 50 per cent of cases. It is difficult to obtain similar data in the United States, but Alice Hamilton[3] reports 358 cases with 16 deaths in 23 white lead factories during the 16 months prior to May 1, 1911. In New York State in 1909 and 1910,[4] 60 deaths were certified from lead poisoning. These figures represent but a small percentage of the individuals who actually suffered from lead poisoning, as the percentage of deaths is relatively low.

In spite of the enormous number of observations made and recorded by various investigators, the mode of action of lead on the body has not been definitely determined. The reason for this is quite evident; being due to the fact that the compounds of lead usually available for experimental purposes, have been, for the most part, such as could not be administered intravenously. Therefore, our knowledge of this subject has been limited mainly to clinical observations.

CLINICAL SYMPTOMS OF LEAD POISONING

Clinically lead poisoning is recognized as being of two types; viz., acute and chronic. The characteristic symptoms of *acute poisoning* develop after the ingestion of a considerable dose of some such salt as "sugar of lead," and usually appear within a few minutes; there is noted immediately a sweet,[5] then a disagreeable metallic and astringent taste, with burning, and a sensation of great dryness in the mouth and throat. Vomiting usually occurs within fifteen minutes, but in rare cases it may be delayed from one to two

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hours. The retching and vomiting are very obstinate, and continue for a long time; the vomitus is often milky with lead chloride and sometimes streaked with blood; there is pain in the abdomen of a colicky character, also thirst. The bowels are as a rule constipated, but occasionally there is diarrhea with black stools (lead sulphide). The urine is generally diminished. The breath has a foul odor, and the tongue is coated; the skin is dry, and the pulse small and frequent. In addition there may be present various nervous manifestations; such as, headache,[6] shooting pains in the limbs, cramping in the legs and local numbness. Fatal cases pass into coma and die with or without convulsions.

The characteristic symptoms of *chronic lead poisoning* are those of general ill health. The symptoms may at first be indefinite, coming on irregularly and often months after the individual has discontinued working with lead. There are general digestive disturbances, loss of appetite, nausea, vomiting, diarrhea or constipation. There are generally present certain blood changes, the number of red cells and the hemoglobin index being low. The red cells, when stained with methylene blue show basophilic granules. This "stippling"[7] is one of the important and almost constant diagnostic signs. Schnitter[8] attributes these blood changes to injury of the bone-marrow.[9] The skin assumes a peculiar yellowish hue, and in some cases the patient is distinctly jaundiced. This is due to the wide-spread destruction of the red corpuscles. The gums show a black line which has been demonstrated to be lead sulphide. The symptoms which often develop later are highly characteristic and assume various forms; in the order of their frequency there may appear colic, arthralgia, paralysis, nephritis, cerebral symptoms and anesthesias. Of Tanqueril's cases [10] there were 1,217 of colic, 101 of paralysis and 72 of encephalopathy.

One of the best pieces of work ever done on experimental lead poisoning was that of Harnack[11] published in 1878. Harnack used lead in organic combination in the form of the triethyl lead acetate, and his observations were made on frogs, rabbits, cats and dogs. The work consisted mainly in administering the lead compound to these animals, either subcutaneously or intravenously, and observing the symptoms which developed. These observations represent a very valuable contribution to our knowledge of lead poisoning, and, considering the date at which the experiments were performed, it is indeed a splendid piece of work. There appear to be, however, many parts of Harnack's work of which further study would be exceedingly desirable. Therefore, I have continued the investigation of this subject, using the following preparations: *Triethyl lead, triethyl lead chloride, triethyl lead acetate, trimethyl lead, trimethyl lead chloride* and *triethyl antimony nitrate.*

PREPARATION OF COMPOUNDS

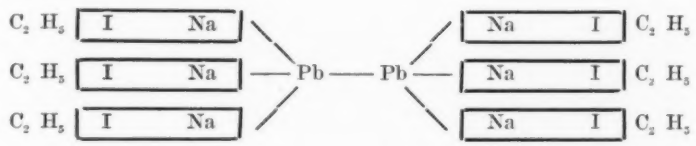
These preparations were first made by Löwig[12] (1853) who prepared the triethyl lead, and in addition the oxide, carbonate, sulphate, nitrate, chloride and bromide of the compound. The work of Löwig was repeated by Klippel[13] (1860) who, in addition, made the following compounds: the phosphate, formate, acetate, butyrate, benzoate, tartrate, oxalate, sulphide, cyanide, sulphocyanate and also double salts with mercury chloride and with platinum chloride.

He also repeated some of the work, using amyl iodide instead of ethyl iodide. As previously stated, Harnack (1878) made the acetate and studied its action on frogs, rabbits, cats and dogs.

I have prepared these triethyl lead compounds according to the method used by Löwig and Klippel with various modifications.

As a starting point for the preparations, it is necessary to obtain a sodium-lead alloy. This alloy is made by melting 50 grams of lead, and introducing into the molten mass 16 grams of sodium, which has been previously cut into rather small pieces, about 1 cubic centimeter in size. The reason for selecting 50 grams of lead is that it gives a yield of a desirable amount, while the reason for combining lead and sodium in a ratio of 50 to 16 is based on their atomic weights (lead 206.9, sodium 23.05), the product desired being an atomic relation of $Pb(Na)_3$.

After the sodium and molten lead are united, a short time should be allowed to elapse until the mass starts to form a crust over the surface. At this point it should be covered with hot, dry sand and allowed to cool. When cool the mass is best ground in an iron mortar, and when reasonably well powdered, it is put in an Erlenmeyer flask of about 200 c.c. capacity. The flask is connected with an upright, water-cooled condenser. Through the upper end of the condenser is introduced 50 c.c. of ethyl iodide or ethyl bromide. A reaction should take place, lasting for two or three hours. If reaction fails to take place, 1 or 2 c.c. of ethyl alcohol may be added. The reaction is as follows:



When the reaction is complete, the excess ethyl iodide or ethyl bromide is distilled off, and the contents of the flask is extracted with ether. This is best done by nearly filling the flask with ether and stirring the contents with a stirring rod. The mixture is then allowed to settle and the ether extract is poured into a graduated cylinder, which should be large enough to contain three such extractions. This extraction process is repeated twice. The extract, after the suspended matter has settled, should be clear and have a greenish-yellow color. It is then poured from the graduate into a distilling flask of one liter capacity, and about 200 c.c. of water is added to the contents. The ether is then distilled off on a water bath, and the compound, being insoluble in water, collects as an oily mass beneath the water in the flask.

This oil has a specific gravity of 1.471 at 10° C., and corresponds to the formula of $(C_2H_5)_3Pb–Pb(C_2H_5)_3$. The compound is but slightly soluble in water, not very soluble in alcohol, but quite soluble in ether.

The salts of the compound may be made by one of two methods; first, by preparing the hydroxide and neutralizing with the acid, or, second, the method introduced by Klippel, which consists in making the desired salt from the carbonate. The first method is the one I have used. The oil is treated in a beaker (with enough alcohol to cover it well) with an alcohol solution of

silver oxide made acid by nitric acid. (The concentrated nitric acid should be diluted with one-half its volume of water, and this nitric acid solution is added until only a small amount of silver oxide is present as such in the alcohol solution.) This alcoholic solution of silver oxide and nitric acid is added to the alcoholic solution of the oil, slowly and with constant stirring until metallic silver no longer precipitates out. The alcoholic solution is then filtered and contains the nitrate of the compound in solution.

$$1\quad \begin{matrix}(C_2H_5)_2\ Pb\\(C_2H_5)_2\ Pb\end{matrix} + Ag_2O = \begin{matrix}(C_2H_5)_2\ Pb\\(C_2H_5)_2\ Pb\end{matrix}\!\!>O + 2Ag$$

$$2\quad \begin{matrix}(C_2H_5)_2\ Pb\\(C_2H_5)_2\ Pb\end{matrix}\!\!>O + 2HNO_3 = 2(C_2H_5)_2\ PbNO_3 + H_2O$$

The solution is then neutralized with an alcoholic solution of sodium hydroxide. A precipitate often occurs at this point, which is probably a basic compound of the lead or silver and should be removed by filtration. The hydroxide of the compound remains in the alcoholic solution.

$$(C_2H_5)_2\ PbNO_3 + \underset{\text{alcoholic}}{NaOH} = (C_2H_5)_2\ Pb(OH) + NaNO_3$$

To obtain the hydroxide in a more purified form, one may treat this alcoholic solution with ether and much water. The water and alcohol are completely miscible which forces the compound into the ether layer. The ether layer is separated from the water by means of a separatory funnel, and the ether layer, after distillation, yields a thick mass, which, on standing, becomes somewhat crystalline.

If the alcoholic solution of the hydroxide is treated with dilute hydrochloric acid, the chloride of the compound will, in the course of time, crystallize in long, beautiful crystals, having a silky luster.

$$(C_2H_5)_2\ Pb(OH) + HCl = (C_2H_5)_2\ PbCl + H_2O$$

In the course of the analysis of these compounds, they were heated, as is the usual custom, to 105° C. to drive off the moisture. However, it was found impossible to obtain a constant weight, and for that reason it was supposed that decomposition was taking place. Therefore, instead of heating samples to 105° C., the temperature was maintained at 95 ° C. The following analysis will show the result of such heating:

Acetate No. III, dried over sulphuric acid for one month, was heated to 95 degrees.

.2476 g. lost .0473 g. or *19.10* per cent.

.2135 g. lost .0624 g. or *29.22* per cent. The heating was discontinued at this point and the samples analyzed.

Analysis of the Dried Acetate.

.2003 g. gave .1708 g. Lead Sulphate or *58.24* per cent.

.1511 g. gave .1283 g. Lead Sulphate or *58.00* per cent. Theoretical *58.62* per cent.

It is evident from the above figures that these compounds undergo sublimation when heated to such temperatures as those indicated. This analysis was made on a preparation which had been prepared in the early part of the

work, and which was not subjected to purification by recrystallization from alcohol. However, the analysis shows a fair degree of purity.

The lead in these compounds is in the masked or protected form, which prevents the lead from reacting with the usual precipitating reagents; such as, chlorides, sulphates, sulphides, and carbonates. Also lead in this combination, when injected into the blood stream, will not precipitate the blood proteins. Therefore, I have been able to inject these compounds intravenously in .5 per cent aqueous solution. The results of such injections are recorded in the following paragraphs.

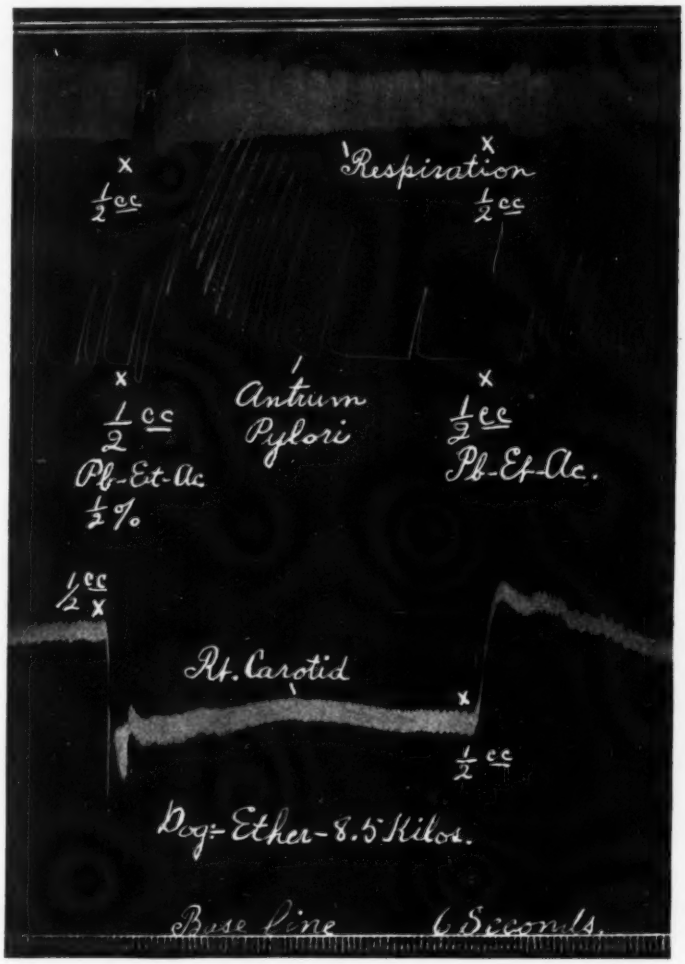


Fig. 1.

EXPERIMENTAL

Fig. 1 shows respiration, pyloric contractions, and blood pressure as recorded in a dog of 8.5 kilos under ether anesthesia. At the beginning (left hand side of the record) there was injected .5 c.c. of a .5 per cent water solution of the triethyl lead acetate. It will be noted that the respiration is completely

stopped. There is a marked increase in tone and activity of the pylorus, and a prompt, pronounced and prolonged fall in blood pressure.

The dose, .5 c.c. of .5 per cent triethyl lead acetate, figured in grams of substance injected, is found to be .0025 grams, or about .0015 grams of lead.

The quantity of lead necessary to produce poisoning is not definitely known. Brouardel[14] states that poisoning may result from 1 mg. of lead per day while others give higher figures. Gaertner[15] claims that symptoms will occur in man only after several months with the daily ingestion of from 4 to

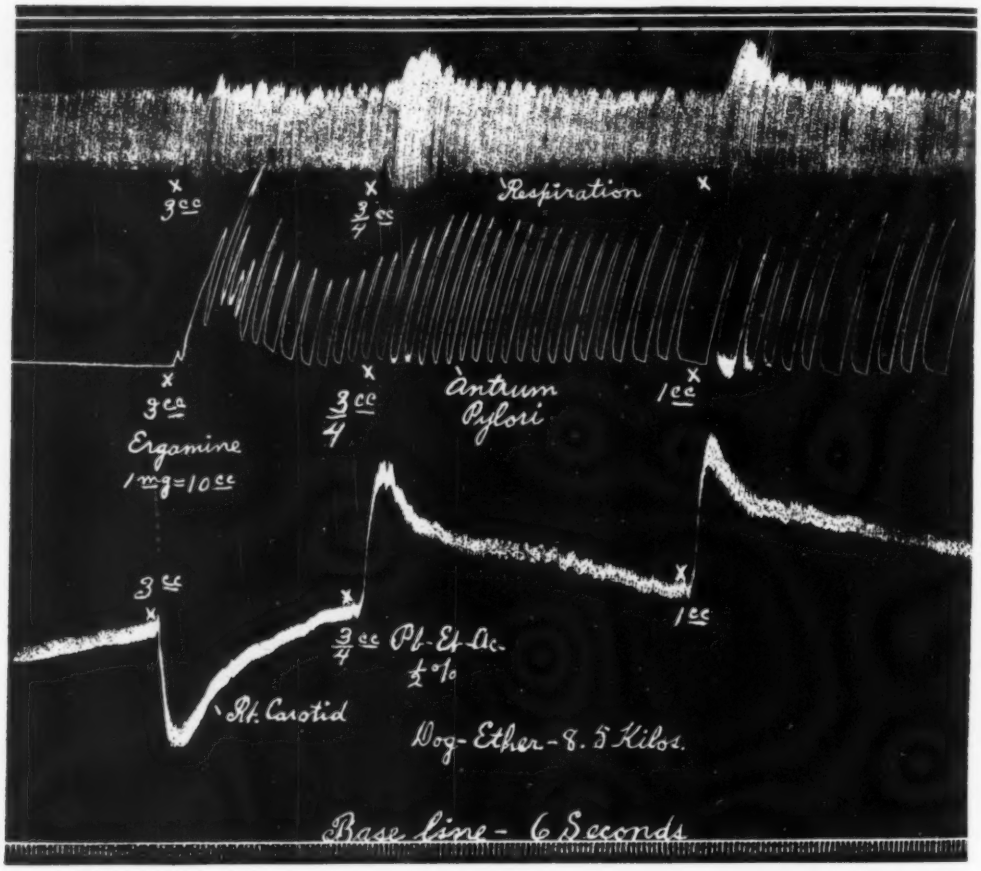


Fig. 2.

7 mg. According to Fluegge and Heffter[16] such symptoms will occur in three or four weeks with the daily ingestion of 60 mg.

The objective symptoms occurring after the first injection of the compound have been recorded by Harnack[17] and are the observations made on an unanesthetized animal. However, they are, for the most part, quite similar to the present observations made on anesthetized animals.

According to Harnack, "the first symptoms following immediately after the injection are doubtless due to the action of the intact compound. The animal yelps loudly and falls in a faint, as if partially narcotized, during which the respiration is completely stopped. If one introduces artificial respira-

tion, the animal will, in the course of a few minutes, breathe and awake from the faint. It is still somewhat dazed and weak and there appears to be psychic irritation, hallucinations, loss of higher functions of the brain, etc. There is also pronounced salivation with a flowing from the nose and a condition of nausea is present; however, without any vomiting."

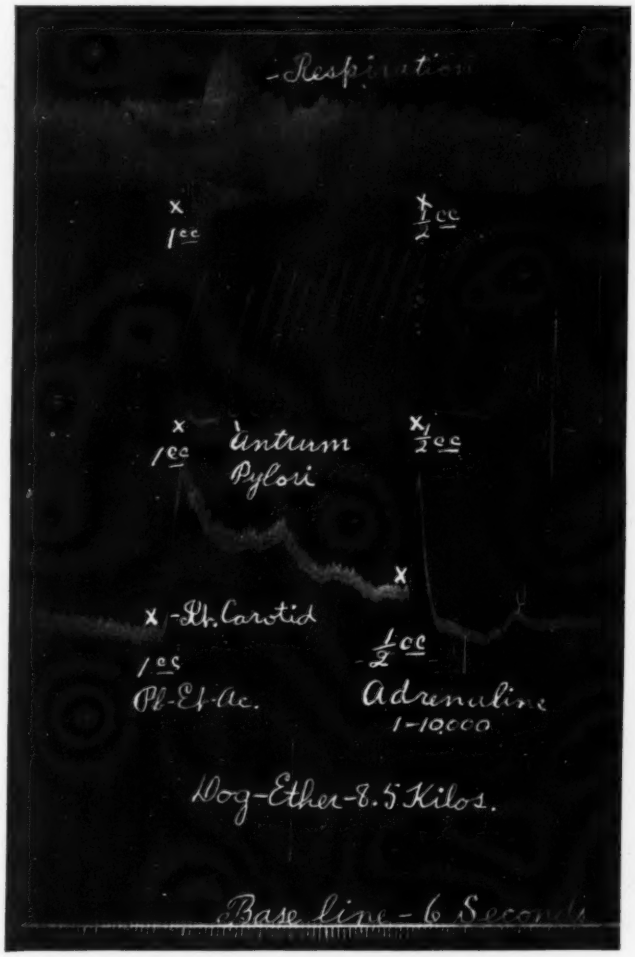


Fig. 3.

It will further be observed in Fig. 1 that the *second injection* of the same amount of the compound is followed by an entirely different result. The blood pressure, instead of falling, rises and remains quite as high as it originally was. Intestinal activity is again somewhat increased and respiration, although poorly shown here, is stimulated instead of being stopped. This striking reversal of conditions apparently completely escaped the attention of Harnack.

Fig. 2 is another record made on the same animal as used in Fig. 1. It shows the result of: first, the injection of 3 c.c. of ergamine solution (1 mg. per 10 c.c.) giving a normal effect for ergamine. The second injection is

¾ c.c. of the .5 per cent triethyl lead acetate solution. The effect on respiration is better shown than in the previous tracing. The third injection of 1 c.c. of the drug, which is the fourth injection of the compound, gives results similar to the previous injection.

Fig. 3 is the third tracing taken from the same animal and shows the result of injecting another cubic centimeter of the triethyl lead acetate solution,

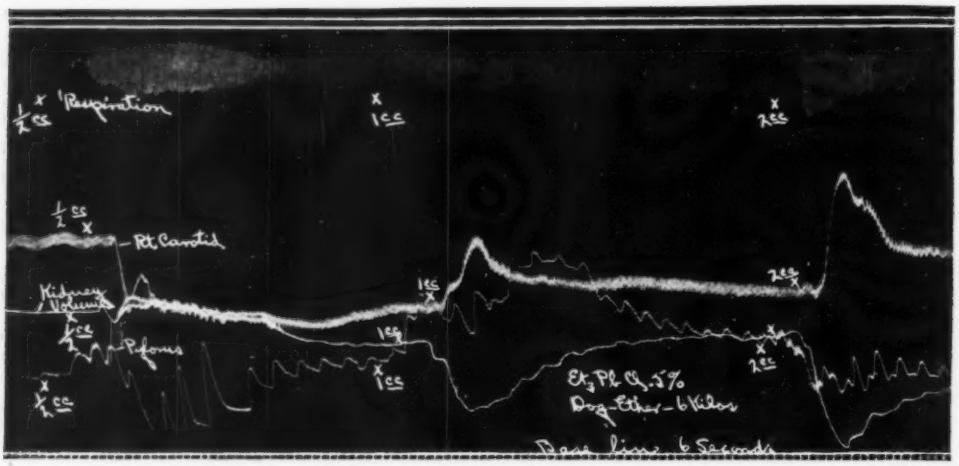


Fig. 4.

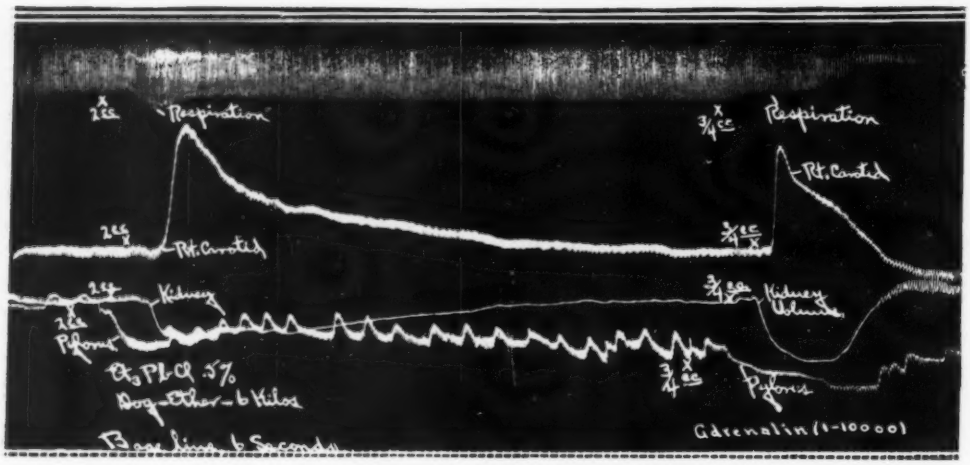


Fig. 5.

the respiration being stimulated, pyloric activity increased, and blood pressure markedly increased. The result of injecting ½ c.c. of adrenalin (1-10,000) is also recorded and furnishes a comparison with the lead compound as well as a check on the technic.

Fig. 4 shows respiration, blood pressure, kidney volume, and pyloric contractions taken on a dog of 6 kilos under ether anesthesia. The first injection of .5 c.c. of .5 per cent triethyl lead chloride gave similar results to those obtained from the acetate salt, the respiration being stopped, blood pressure fal-

ling, and pyloric tonus and activity increased. In addition, it will be seen that the kidney volume, although but slightly altered, did show the heart beat much more plainly than before the injection. The second injection of 1 c.c. of the drug showed practically the same results as those obtained from the second injection of the acetate, with the additional fact that the kidney is constricted. The third injection of 2 c.c. of the chloride shows the stimulation of the respiration and the marked rise in blood pressure, also a decrease in kidney volume.

Fig. 5 is a second record made from the same animal and shows the result

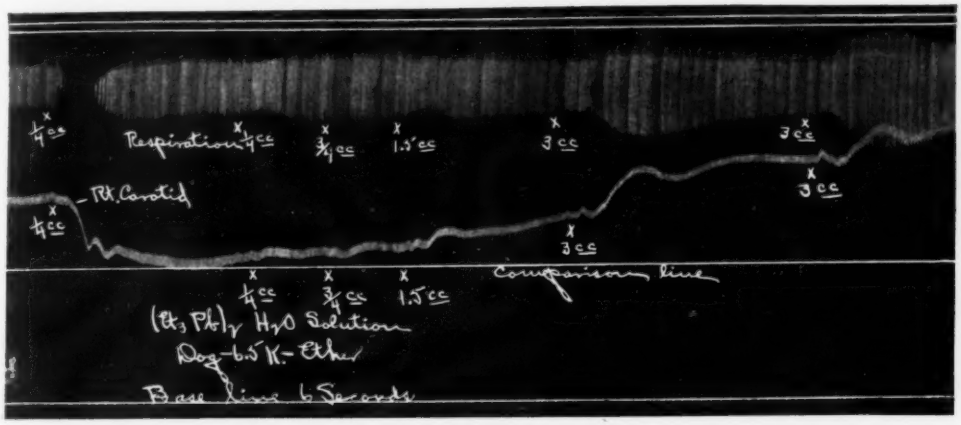


Fig. 6.

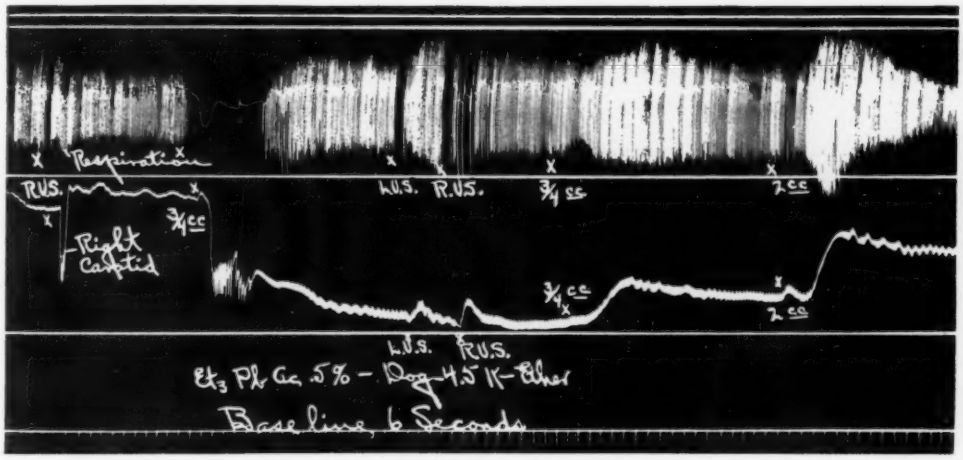


Fig. 7.

of the fourth injection of the chloride, and at the right hand side of the record is shown the result of injecting ¾ c.c. of 1-10,000 adrenaline.

Fig. 6 is a record made on an animal of 6.5 kilos under ether anesthesia, and shows the respiration and blood pressure with the result of injecting an aqueous solution of the triethyl lead. The water solubility of this compound has not been determined exactly; however, it appears to be but slightly soluble. The first injection of ¼ c.c. of such an aqueous solution gave a very similar result to those obtained from the use of the salts previously discussed.

With additional injections, the reversal of the first results did not occur so promptly, but this might be due to the size of the doses administered, and not to any marked difference in their action.

The characteristic fall in blood pressure following the first injection and the rise following the second injection, would suggest the possibility that the vagal action upon the heart was involved in producing the first fall and that

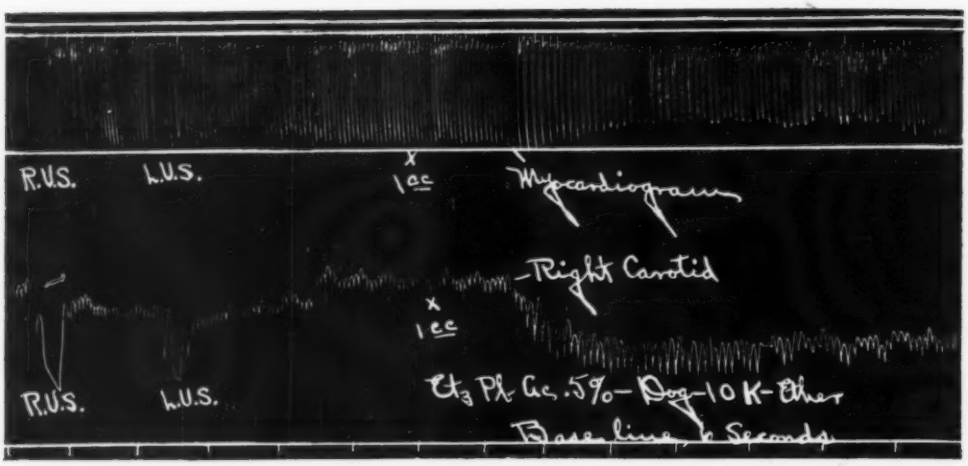


Fig. 8.

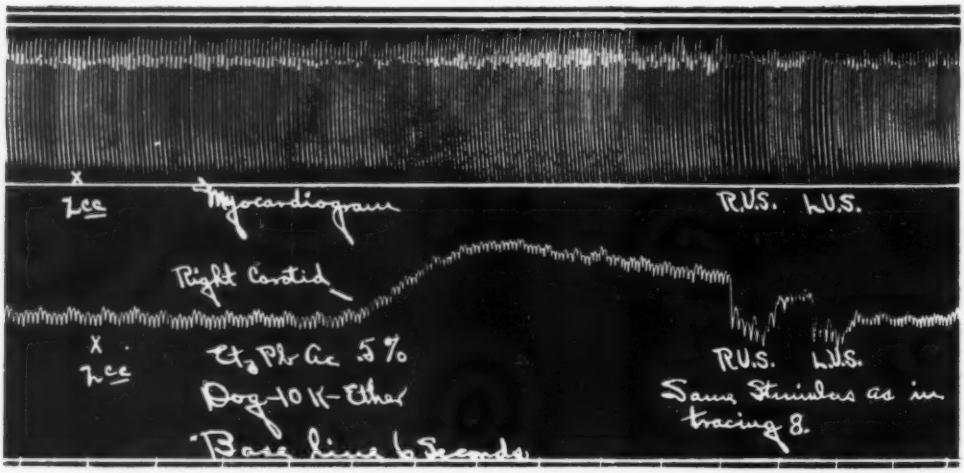


Fig. 9.

such action was not present at the time of the second injection. Fig. 7 is a record which apparently supports this suggestion. It shows the respiration and blood pressure in a small dog of 4.5 kilos under ether anesthesia. At the left side of the record at the point marked R.V.S., the right vagus was stimulated in the neck and the characteristic results of such stimulation are recorded. There was injected ¾ c.c. of triethyl lead acetate solution, and after about two minutes the left vagus was stimulated, causing a stoppage of respiration and an asphyxial rise in blood pressure. However, the vagal stimulation

showed no direct action on the heart. Stimulation of the right vagus gave similar results. These results would lead one to believe that the first action of the drug on blood pressure is due to vagal stimulation followed by paralysis.

Harnack, in discussing the action of the triethyl lead acetate on the rabbit, says:[18] "Also the heart muscle and the muscles of respiration take part in the paralysis, as is indicated by the weak pulse, the slow superficial respiration, the lowering of temperature of the peripheral parts and the weak filling of the blood vessels in the periphery of the body." Desiring to determine whether or not such a weakening of the heart muscle was responsible for the lowered blood pressure in the *dog*, I made myocardiograph records such as is shown in Fig. 8 which is a record of the blood pressure and direct heart muscle action.

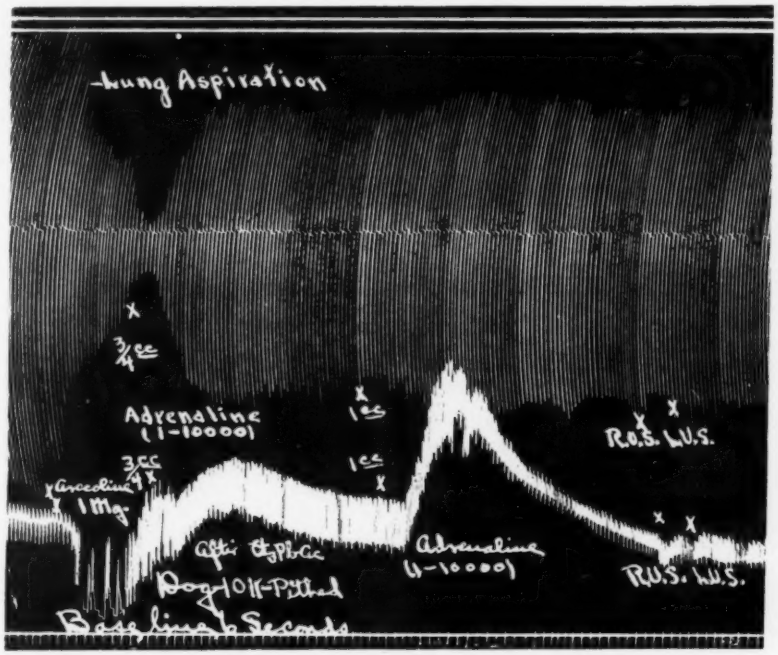


Fig. 10.

At the left side of the record, the right vagus was stimulated. This produced a slowing of the heart with a fall in blood pressure. Stimulation of the left vagus gave similar results. After a normal blood pressure was again established 1 c.c. of the .5 per cent triethyl lead acetate was injected. It will be noted that about 6 or 8 seconds later there occurred the characteristic fall in blood pressure, which was accompanied by a slowing and a weakening of the heart beat. Fig. 9 shows the result (taken on the same animal as was used in record 8) of injecting 2 c.c. of the solution. In about 24 seconds the blood pressure rose and the heart beat was more rapid and stronger. Stimulation of the right and left vagus, with the same strength stimulus as used in record 8, showed the vagus was still active, but not so strongly as in Fig. 8.

At this phase of the work it was thought important to determine whether the innervation of the bronchial musculature was also affected similarly to

that of the heart. Fig. 10 shows the result of such observations. This animal was pithed and, after receiving 20 c.c. of triethyl lead acetate solution, 2 c.c. at a time, the record (Fig. 10) was made. The first injection at the left was 1 mg. of arecoline. The normal action of arecoline on the lung volume and blood pressure is observed. After constriction of the lungs by the arecoline, ¾ c.c. of adrenaline 1-10,000 was given with the result that adrenaline dilated the lungs in the normal manner. Another c.c. of adrenaline was again injected, which produced a further dilatation. It will be noted that vagal stimulation (R. V. S. and L. V. S.) had no direct action on the heart. It would appear from this record that the drug does not paralyze the endings of the postganglionic fibers of the vagi, or of the sympathetics in the lungs.

In attempting to determine whether or not the sympathetic ganglia are acted upon by the drug, a series of experiments were carried out, using lobelin.

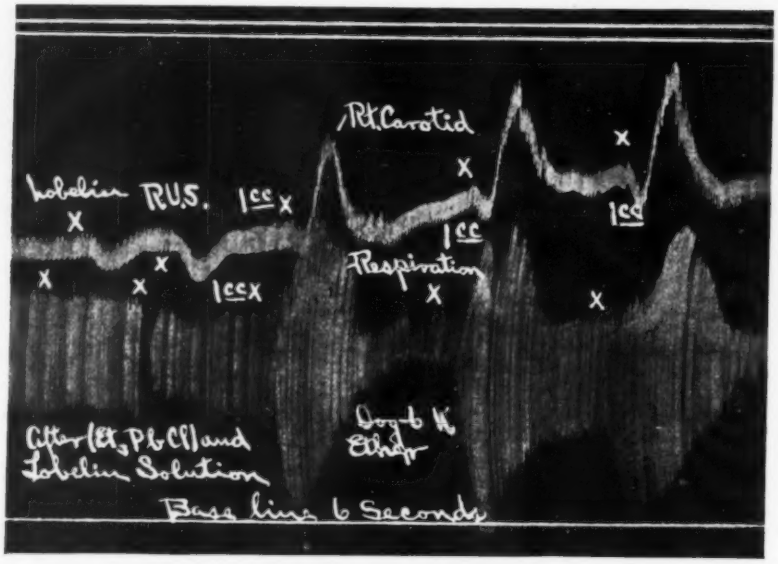


Fig. 11.

Lobelin is a drug which first stimulates and later paralyzes the sympathetic ganglia,[19] an action quite similar to that of nicotine. Fig. 11 shows a record taken on an animal which had previously received three or four injections of triethyl lead chloride, and had given a normal response to each. A small amount of lobelin solution was then injected and the usual reaction, due to stimulation of the sympathetic ganglia, was observed. The record shows the result of a second injection of lobelin, which by the lack of response, indicates that the ganglia are paralyzed. As a further check, the right vagus was stimulated with the result shown. It will be observed that, after the administration of lobelin, the results of administering triethyl lead are very similar to those obtained from a normal animal. In answer to the question as to whether or not a primary fall in blood pressure, following the first injection of triethyl lead salts is obtained after lobelin, we may refer to Fig. 12. This tracing shows the respiration and blood pressure as recorded in a dog

of 8.5 kilos under ether anesthesia. The blood pressure changes in this particular animal, following the injection of lobelin and triethyl lead solution, are not very pronounced. However, they are completely typical, and the record serves to show that the triethyl lead acts in its usual manner after the injection of lobelin.

Harnack, in discussing the fall in blood pressure, produced in rabbits, maintains that the vessel walls are not acted upon, and that the vasomotor center is not affected, but that the whole action is due to the weakened condition of the heart. As evidence he submits the following:[20] "To decide this question, the following course of research was carried out: a rabbit which showed the action of triethyl lead decidedly, was, while in such condition, examined to note the state of the ear vessels. Such animals, if then subjected to chloro-

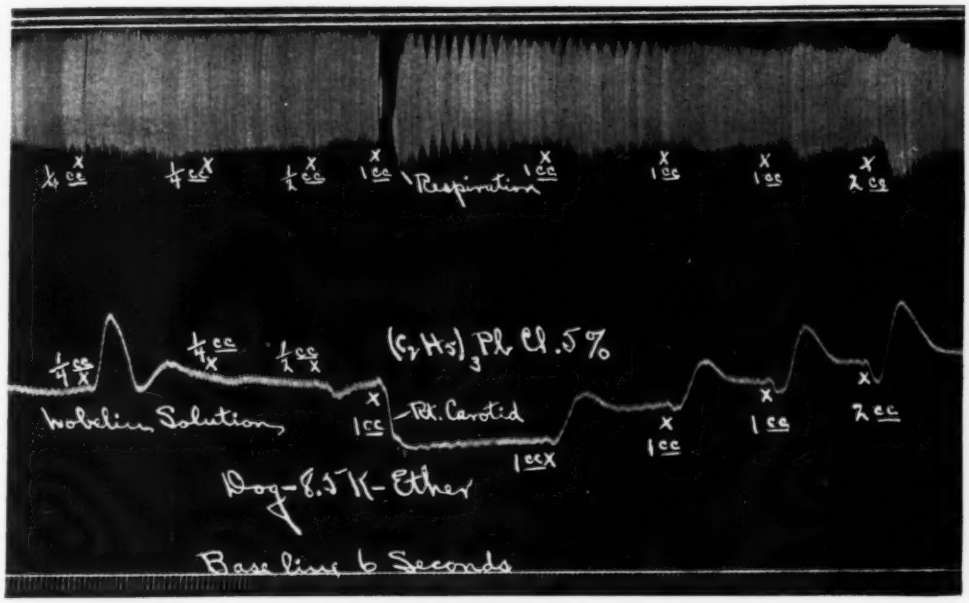


Fig. 12.

form anesthesia, showed a widening and filling of the ear vessels, which were previously narrow and weakly injected. This shows that the vessel muscles are not directly stimulated by the lead." In order to show that it is not the vasomotor center which is affected, he carried out the following experiment. In a rabbit (1570 grams) he cut the sympathetic nerve on the right side of the neck and, after the vessels in the right ear dilated and increased in temperature, he injected .040 grams of triethyl lead acetate subcutaneously. The following morning there was only a slight beginning of paralysis. Therefore, another small quantity of the salt was given (amount not stated). Very soon the paralysis became greater, the heart beat weak, and the animal cool. The vessels in both ears were bloodless, and those in the right remained wider than in the left. Chloroform was then given, and the left ear vessels dilated. The animal died during the anesthesia from "heart paralysis." There were therefore no grounds, he decided, for supposing that the lead stimulated the vaso-

motor center in rabbits, or that the irritability of the vessel muscle was increased.

I have studied a series of animals in order to determine whether injec-

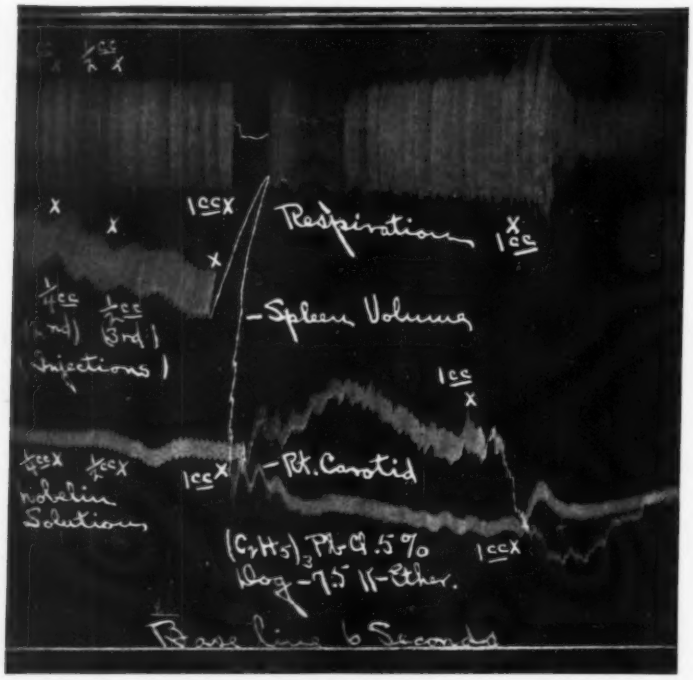


Fig. 13.

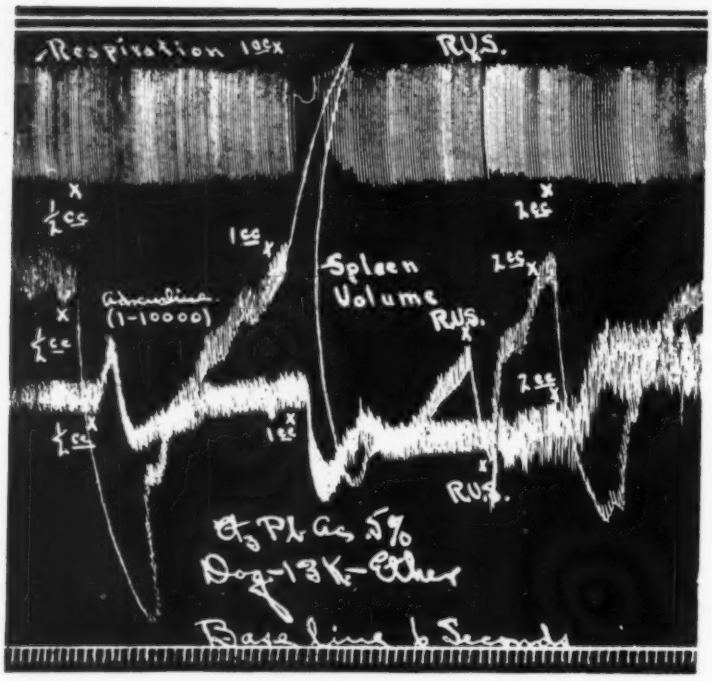


Fig. 14.

tion of the drug produced any volume change in the spleen or kidney. Fig. 13 is a record taken in the course of such work and shows respiration, spleen volume and blood pressure. The first two injections, shown on the record, are those of lobelin sulphate solution and are the second and third injections. It will be noted that the lobelin had no noticeable action, which means that the first injection (not shown in the tracing) was sufficient to paralyze the sympathetic ganglia. The injection of 1 c.c. of the .5 per cent triethyl lead chloride gave complete stoppage of respiration, an initial increase in spleen volume followed immediately by a decrease, and the blood pressure was lowered. The second injection of the drug stimulated respiration, decreased spleen volume, and increased blood pressure.

Fig. 14 shows respiration, spleen volume, and blood pressure. The injection of ½ c.c. of 1-10,000 adrenaline solution gave the characteristic action

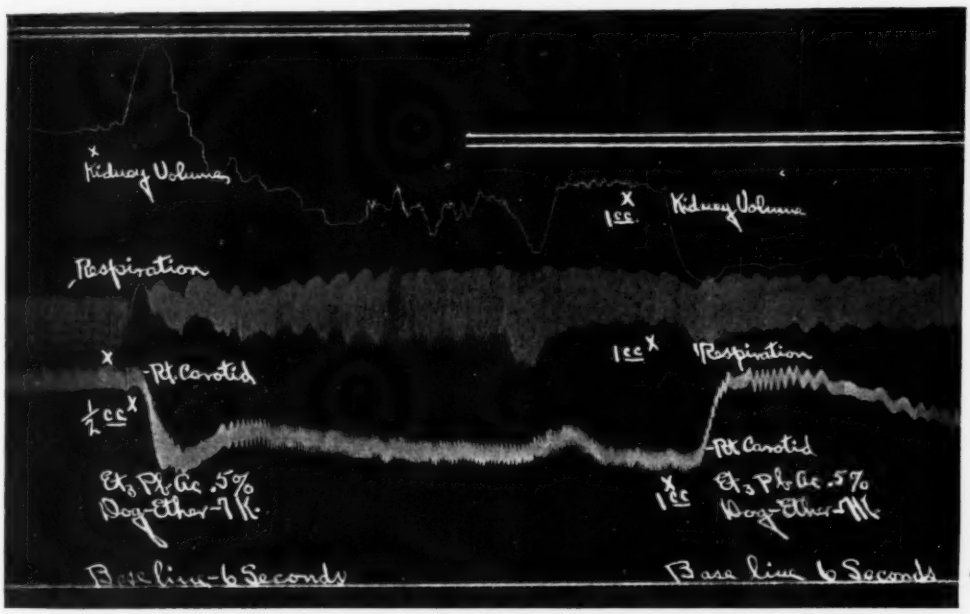


Fig. 15.

on the spleen volume and some increase in blood pressure. The injection of 1 c.c. of a .5 per cent solution of the lead salt gave stoppage of respiration, an increase in spleen volume followed immediately by a decrease, and the blood pressure fell as usual. The principal thing of interest in the second injection of lead (2 c.c.) is the marked contraction of the spleen *without the preliminary dilatation.*

Fig. 15 shows kidney volume, respiration and blood pressure. It will be noted that with the first injection the kidney volume increased. This increase in volume is followed by a not very marked, but prolonged decrease. The second injection of the drug gave only the decrease in kidney volume.

Fig. 16 is presented to show the initial increase in kidney volume, followed immediately by a pronounced decrease in volume. It will also be noted that, with this first injection of the drug, there is a marked increase in tonus activity

of the pylorus. In this connection, reference to Fig. 1 will show that intestinal activity was there also markedly increased.

In order to determine whether or not the vagus endings are the seat of action of the lead salts, the triethyl lead acetate was administered after atro-

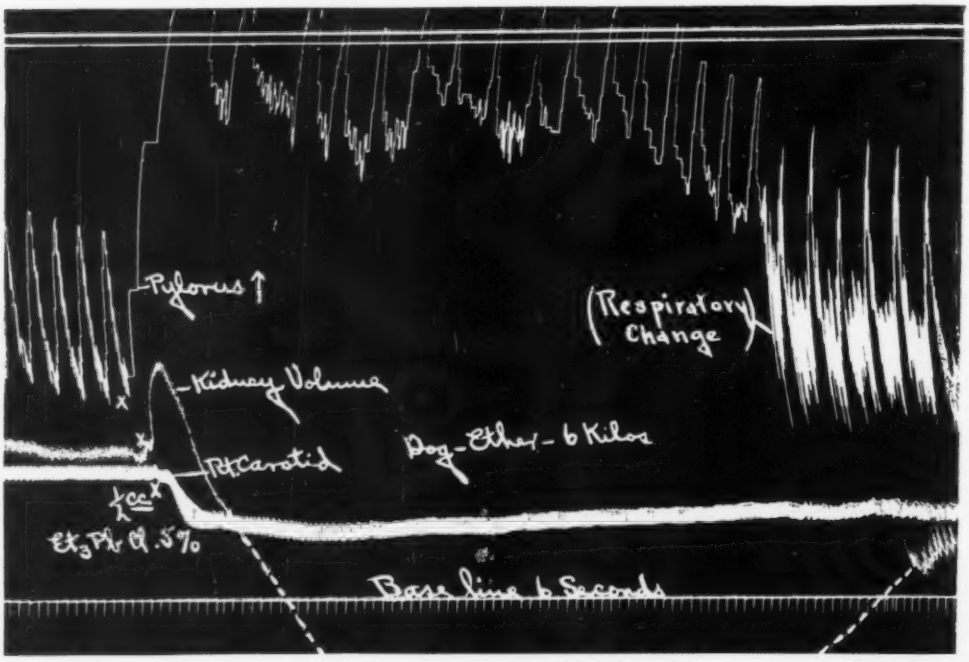


Fig. 16.

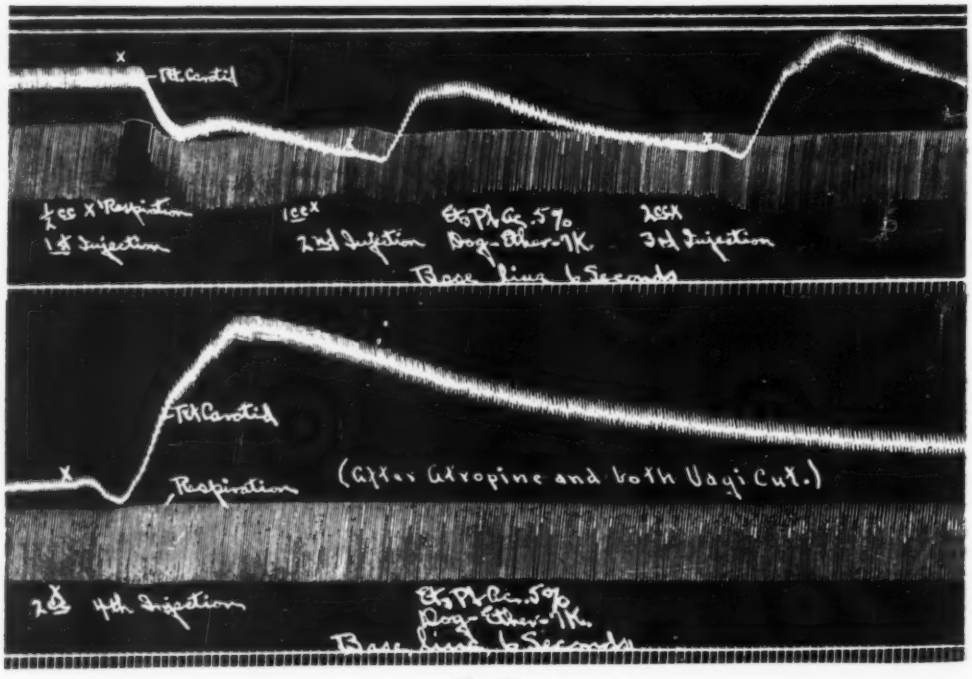


Fig. 17.

pine, and also after atropine and section of both vagi. Fig. 17 is a record of blood pressure and respiration recorded in an animal after the administration of atropine and section of both vagi. It will be noted that the first injection (*upper half of tracing*) produced a cessation of respiration and a fall in blood pressure, somewhat similar to the effect observed on animals with intact vagi; also the subsequent injections of the triethyl salt gave results similar to those obtained from normal animals. It is of interest to note the relative height and duration of the second and fourth injections.

Fig. 18 shows lung volume and blood pressure as recorded in a pithed dog of 6.5 kilos. Following the injection of .5 c.c. of .5 per cent triethyl lead chloride solution, the lungs show a slight but insignificant increase in volume. The blood pressure was gradually somewhat lowered and remained at a constant level regardless of the later injections of 1, 2 and 4 c.c. of the

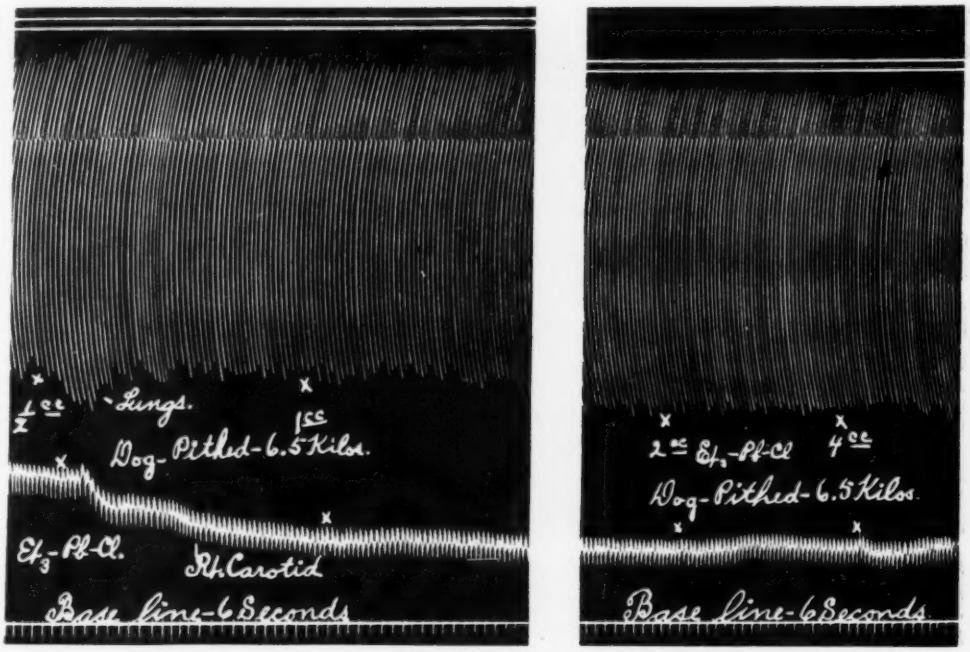


Fig. 18.

drug. It is evident that the drug has but little apparent action on a pithed animal. There are those who maintain that the dyspnea accompanying lead colic, is due to a spasm of the bronchial muscles.[31] However, it will be observed that lead, in such a form as here injected, does not cause a constriction of the bronchioles, but, if anything, a slight dilatation.

In order to further localize the seat of action on the central nervous system, the cord was sectioned below the origin of the phrenic nerves and the drug was then injected. Fig. 19 shows a record of such an experiment. The animal was breathing normally, as is shown by the record, and it will be observed that the respiration was greatly influenced by the drug, but that blood pressure shows only slight changes; the initial rise is possibly due to the action of the diaphragm. The injections following the first showed no significant ac-

tion on the blood pressure. Fig. 20 is a record made on a dog in which the cord was sectioned at the seventh cervical and both vagi divided. The vagi were sectioned at the two crosses marked on the tracings as V. S. Following the section of the vagi, 1 c.c. of the acetate of the usual strength was injected. The respiration was stopped as usual, and it was found necessary to administer artificial respiration between the points marked with crosses as indicated.

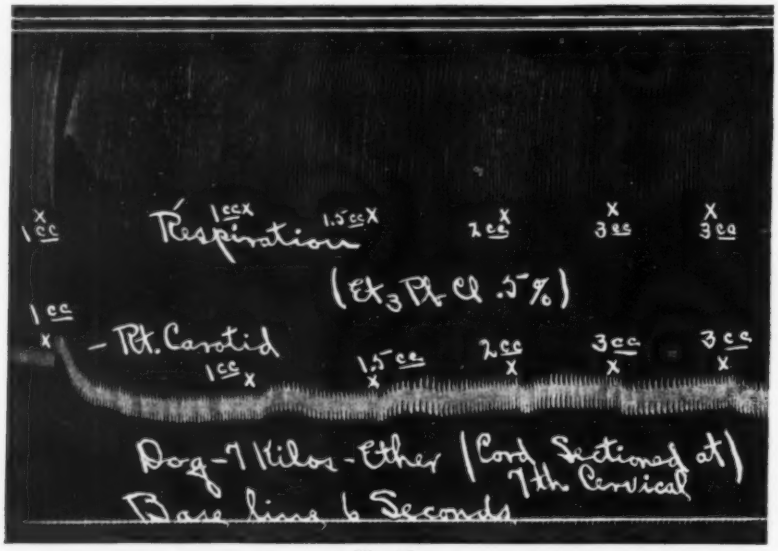


Fig. 19.

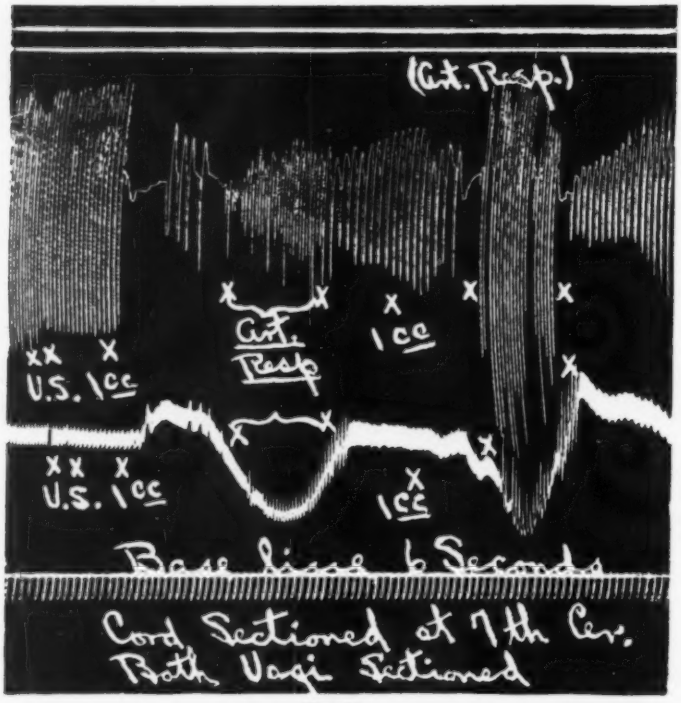


Fig. 20.

The most interesting part of the record is that the first injection produced a slight rise in blood pressure, and not a fall. The second injection of 1 c.c. did not alter the blood pressure, but did affect the respiration, making it necessary to introduce artificial respiration.

Desiring to determine whether the activity of the compound is due to the particular salt, or to the lead in the molecule, or to the special arrangement

Fig. 21.

of the ethyl radicals in the molecule, I extended the work to include the preparation and use of the trimethyl lead, trimethyl lead chloride, and triethyl antimony nitrate. That the action is not due to the particular salt of lead triethyl is evidenced in the work already presented.

The preparation of trimethyl lead is described by Cahours;[21] however, I prepared it in much the same way as the ethyl compounds, and for the chloride I obtained a preparation of white, thin, crystalline plates, having an odor not unlike the methyl amines. I did not run an analysis on the product, and for that reason, am not certain as to its absolute purity; however, as a check on the work, I used an aqueous solution of the oil, which is $(CH_3)_3Pb–Pb\ (CH_3)_3$ and later a 50 per cent alcoholic solution of the compound. The oil must necessarily be of a rather high degree of purity.

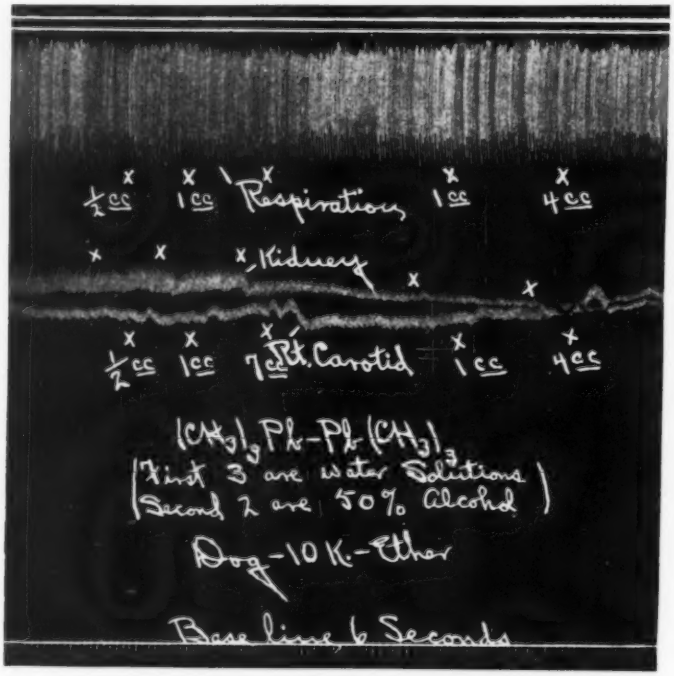

½ cc 1 cc Respiration 1 cc 4 cc
Kidney
½ cc 1 cc 7 cc Rt. Carotid 1 cc 4 cc
(CH3)3 Pb-Pb (CH3)3
(First 3 are water Solutions
Second 2 are 50% Alcohol)
Dog-10 K.-Ether
Base line, 6 Seconds

Fig. 22.

Fig. 21 is a record of blood pressure and respiration taken on a dog under ether anesthesia. The first injection is that of 2 c.c. of .5 per cent *trimethyl lead chloride*. There is no apparent change in the respiration, and the blood pressure shows only a slight fall immediately followed by a greater rise. The next two injections of 7 and 15 c.c. correspond quite closely to the first injection. The injection of 1 c.c. of triethyl lead chloride solution at this point gave the characteristic fall in blood pressure and a stoppage of respiration, followed after a brief interval by a pronounced stimulation. The second injection of 1 c.c. of triethyl lead salt acted in the usual manner. The next two injections of 7 and 15 c.c. are of the methyl compound and show nothing very striking. The last injection of the ethyl compound gave a very pronounced result.

As a further check, the aqueous solution of the trimethyl lead was used. It will be noted in Fig. 22 that such a solution did not show any action on respiration or blood pressure. The kidney volume did show that filling of the kidney was not so good as time went on; this may be due to the action of the compound. Not knowing the solubility of the compound in water, a saturated solution in 50 per cent alcohol was used, which shows no pronounced changes. Fig. 23 is another record taken on the same animal as used in Fig. 22. It will be noted that a water solution of the triethyl lead was very active. The third injection of 3 c.c. proved to be a fatal dose.

These records imply that either one or the other of two things is responsible for the activity of triethyl lead compounds; either that the lead radical in that particular combination is responsible; or that the activity is due to

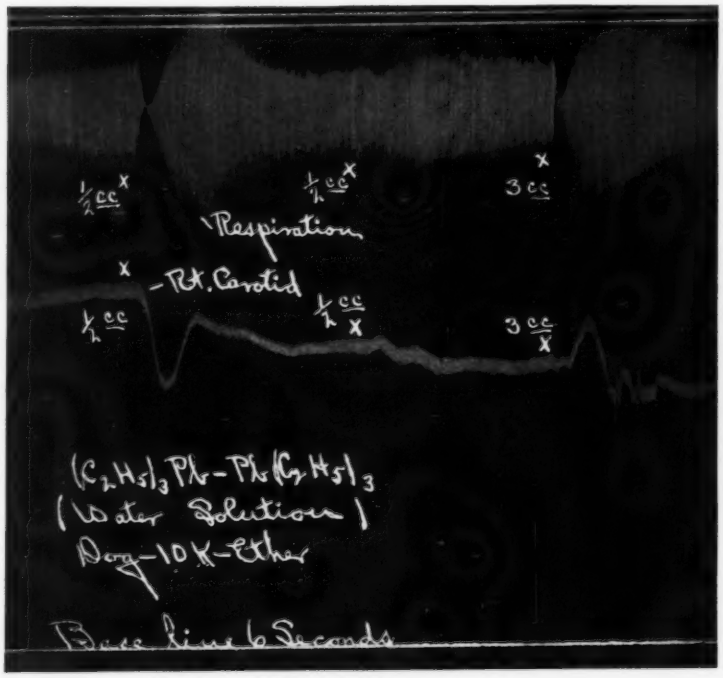


Fig. 23.

the peculiar combination of the ethyl radicals with the lead. Apparently trimethyl lead does not act in the body as the triethyl lead compounds do. This point is not so startling when one considers that there are many substances which differ chemically much less than triethyl lead and trimethyl lead differ (for example, some of the sugars), but which, when taken into the body, vary greatly in their assimilability by the tissues (as occurs with levulose and dextrose).

To determine whether the activity of the triethyl compounds is due mainly to the particular grouping of the ethyl radicals, I have prepared and used *triethyl antimony nitrate.* The triethyl stibine is obtained by the action of ethyl iodide on an alloy of antimony and sodium in a manner similar to that used in the preparation of the triethyl lead.[22] It is a colorless liquid having an

odor of onions, and on coming in contact with the air, oxidizes instantly and burns with a white flame. Triethyl stibine oxide is obtained by shaking an alcoholic solution of the triethyl stibine with finely divided mercuric oxide. Mercury is set free, and the oxide of the radical is produced.

The nitrate can be prepared by: First, the action of nitric acid on the triethyl stibinic oxide; or, second, the action of dilute nitric acid on the triethyl stibine. The nitrate crystallizes out in well defined, rhomboidal crystals with a melting point of 62.5° C. It is very soluble in water and has a bitter taste.

Fig. 24 shows respiration and blood pressure as recorded in an animal of 10 kilos under ether. Triethyl antimony nitrate was injected in a 1 per cent water solution. It will be noted that the results of the injection in no way correspond to those obtained through the use of the triethyl lead salts. It is

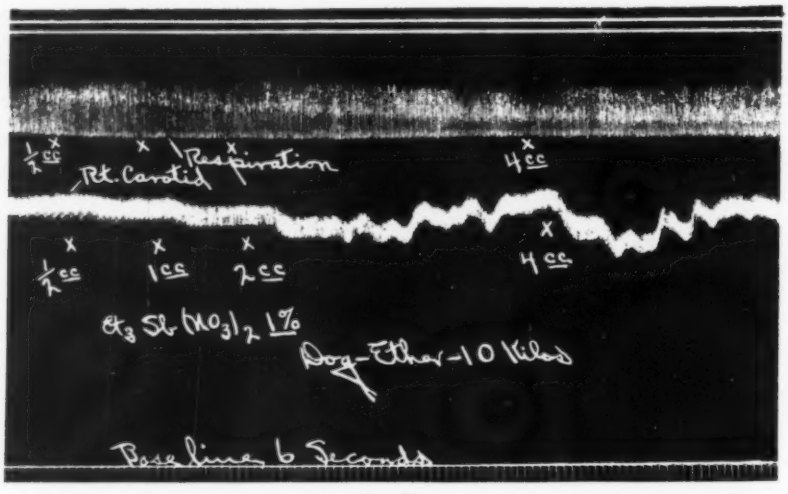


Fig. 24.

of interest that, after this tracing was made, the lead salt was injected and the characteristic results obtained. From this, it appears that the ethyl radical is not chiefly responsible for the activity of the triethyl lead compounds.

EXCRETION

Lead is excreted mainly by the feces (small and large intestine and bile) and to a minor degree in the urine; also traces appear in the saliva, sweat and milk.[23] After ingestion all is excreted in organic combination, except the unabsorbed fraction in the feces.[24]

Dauwe[25] states that lead injected intravenously disappears rapidly from the blood, the greater part disappearing in less than two minutes. Kobart[26] claims that the main deposition of lead in the body occurs in the kidneys, the bones, liver and other glands, a minor amount in the brain, striped and smooth muscle, and blood.

In attempting to determine the mode and rate of excretion of the triethyl lead compounds, Harnack failed,[27] after numerous attempts, to show the presence of any undecomposed triethyl lead in the urine of animals poisoned with the compound. He maintained that, if such a radical should be present,

it would be easy to isolate it and to demonstrate its presence. He presents the following data obtained from large samples of urine, collected for long periods of time:

Three and one-half liters of urine, collected from a rabbit which had received during the period (45 days) a total of 0.3 grams triethyl lead acetate administered subcutaneously, gave a yield of .0077 grams of lead. There was therefore about 4 per cent of the lead excreted in the urine. The feces were not analyzed.

In the present work, I have, on several occasions, tested for the presence of lead in the samples of urine obtained from the animal at the end of the experiment. These animals would often receive a total injection of .125 grams during the experiment, and occasionally as much as .250 grams have been given, the duration of the experiment often being two hours or more. However, I have not, in any case, been able to detect the lead in the urine, either by applying the test to the urine directly, or by evaporating it to dryness in the presence of concentrated nitric acid and testing for the lead salt. However, I do not maintain from these findings that lead is not to a certain degree excreted in the urine.

DISCUSSION

With the foregoing experimental data at hand, it is very interesting to review the conclusions formulated by Harnack. He considers that interpretation of his results justify the following conclusions,[28] translated somewhat freely:

(1) That lead affects the substance of all cross striated muscle; not that it primarily makes each contraction impossible, but that it causes the active muscle to become quickly exhausted; also the muscle finally loses its irritability and dies; yet the lead does not interfere with rigor mortis. This action of lead is more pronounced in the frog and rabbit than in other animals.

(2) That lead affects certain central motor nerve mechanisms; the action consists of a stimulation, from which originate peculiar ataxic movements, also a continuous trembling and twitching, and finally convulsions; yet, consciousness remains and sensibility is not lost. The seat of action is probably the mid-brain or cerebellum. The action is especially marked in the dog, but is also noted in cats and pigeons.

(3) That lead irritates certain nerve structures, located in the intestinal wall, which control the intestinal movements. This stimulation therefore causes general contraction and increased peristalsis of the intestines, producing colic and causing an increased irritability of the whole belly, also diarrhea. The action is clearly outspoken in all mammals which were studied. An action on the smooth muscle of the intestine, of the blood vessels, etc., is not demonstrable. Respiration and circulation are not directly influenced, except that in animals in which the muscle paralysis is very prominent, then also the heart and respiratory muscles take part in the paralysis.

Although the records which I have presented do, to a certain degree, confirm some of Harnack's observations, there are, however, many points on which we differ widely. One of the most striking differences deals with the questions of respiration and circulation. Harnack was of the opinion that these were not primarily affected; however, there seems to be no doubt that, as

shown by my experiments, both respiration and circulation are greatly affected by the administration of the triethyl lead compounds. To determine the manner in which respiration and circulation are affected has been the object of a great deal of the present work.

It is generally assumed that chronic lead poisoning is accompanied clinically by a rise in blood pressure and vasoconstriction. Riegel and Frank[29] stated that, during the attacks of colic, the blood pressure was increased, and it has therefore been suggested that the hypertension may be due to the violent contraction of the intestines which produce mechanical displacement of blood, or to the pain reflexes. The pallor of the skin has been considered an indication of vasoconstriction. Quellien[30] believes that even in acute poisoning the blood pressure is raised.

That the blood pressure changes as produced with lead triethyl salts are not due to the increased activity of the intestines with the forcing out of the blood from the intestinal area is clearly shown in Fig. 1 and again in Fig. 16, in which it will be observed that, regardless of the violent intestinal activity produced by the injection of the first dose, the blood pressure falls and remains low until the second injection of the compound. At this time peristalsis is not any more active, if even as vigorous, as following the first injection; yet the blood pressure is strikingly increased.

That the blood pressure changes are not wholly due to muscular activity resulting from central nervous stimulation is evident, for there is present as much or more, muscular activity following the first injection as there is after the second; yet, a fall in pressure is produced by the first injection instead of a rise. Also, if an animal is deeply under anesthesia so that no muscular activity is observed, the fall and rise are produced in the usual order.

That the cessation of respiration in the first case and the stimulation in the second are not directly responsible for the blood pressure change is especially well shown in Fig. 17 and others, where it will be observed that the intensity of the blood pressure change does not, in any degree, correspond to the respiratory changes.

One factor which appears to be quite active in producing alterations in the blood pressure is the variation produced in the rate and volume output of the heart following the first and subsequent injections. This point is shown in Figs. 8 and 9. After the first injection of the compound, the heart beats more slowly and the amplitude of the beat is considerably less. This action appears to be partly due to stimulation of the vagus. But Fig. 9 shows that, following the second injection, the heart rate and amplitude are considerably increased. It appears that the cardiac vagal action present at first has disappeared, and it was found in several animals, the tracing from one of which is shown in Fig. 7, that stimulation of the vagus with the electric current was either without action, or the action was much less, after the administration of the drug. From the records presented one is likely to doubt this proposed explanation of vagal stimulation and paralysis, since Fig. 17 was obtained after atropine and Fig. 13 after lobelin, and both show changes somewhat similar to those obtained from animals with intact vagi. However, a more careful consideration will show that the fall in blood pressure in both

of these cases was not a sudden fall such as results from electrical stimulation of the vagus, or, as that observed in Figs. 1 and 7.

The sudden dilatation of the spleen and kidney, following the first injection might also play some part in the fall of blood pressure, while the constriction of these organs evidently plays an important part in the rise following the subsequent injections.

Animals, injected with the triethyl lead compounds, unless deeply under anesthesia, showed a marked stimulation of the higher centers, at times the stimulation being so intense that the animal would wink its eyes and apparently regain consciousness, although it was completely under the anesthetic just at the time of injection. These results and the violent action on the respiration prove that the drug acts on the higher centers and the medulla.

It is very interesting that triethyl lead and its compounds should be so active in stimulating the higher centers and the medulla, for it would be expected that, if the ethyl radicals exerted any action, this would be in the nature of depression, for such is the property of most of the ethyl compounds; for example, ether, ethyl bromide, etc. And again, it would scarcely be expected from the general character of chronic lead intoxications that the metal possessed strongly stimulating properties; therefore, it is rather surprising to find the compound so extremely active.

This violent stimulation of the medulla and basal ganglia is, beyond all doubt, responsible for most of the symptoms resulting from the administration of lead in such a combination. It will be observed that the drug has practically no action on blood pressure when administered to a pithed animal. Also in a dog with the cord sectioned at the seventh cervical vertebra, the result is practically the same, while after section of the cord and both vagi, the blood pressure shows a slight, but insignificant rise.

A pithed animal (brain and medulla), or an amimal in which the cord has been sectioned at the seventh cervical vertebra shows no signs of convulsions. This means that the medulla and possibly the higher centers are responsible for the convulsions.

The violent stimulation of the respiratory center in the medulla explains the changes produced in respiration. It seems possible that the stoppage of respiration following the first injection is due to extreme stimulation of the center, (or, of some higher controlling and inhibiting mechanism,) and that the increased respiration following subsequent injections is the result of direct stimulation of the center itself after its normal hypersusceptibility to the compound has been slightly depressed by the first injection of the substance.

The kidney and spleen volume changes also strongly suggest stimulation of the vasomotor centers. The primary dilatation is apparently of central origin. But I have suspected many times that, in addition to this action, there might be some peripheral mechanism concerned, possibly in the nature of a brief primary stimulation of vasodilator nerve endings. The marked shrinkage in volume of the kidney and spleen which comes on secondarily is certainly due mainly, and perhaps entirely, to stimulation of the medullary vasoconstrictor center. It is exceedingly probable that this same action is the

cause of the prolonged hypertension which characterizes chronic lead poisoning.

There is also a very strong probability that the increased intestinal activity, which gives rise to colic and other intestinal disturbances, including constipation, following the ingestion of lead is of central origin. And the slow pulse of chronic lead intoxication may very well be due partly, and perhaps mainly, to stimulation of the medullary inhibitory center.

While in the early part of this work I was inclined to suspect that the sympathetic ganglia might be the seat of action of the metal in the production of various phenomena, especially in regard to the effects on intestinal peristalsis; yet, in the long run I have been unable to find any positive evidence that the ganglia are directly affected. In Fig. 19, for example, it was found that repeated injections of triethyl lead chloride produced no rise whatever in blood pressure after section of the spinal cord at the level of the seventh cervical vertebra. If the vasoconstrictor ganglia had been stimulated in this case, as would have occurred under nicotine the blood pressure should have risen as in the normal intact animal.

CONCLUSIONS

(1) Triethyl lead and salts of triethyl lead are extremely active in stimulating the central nervous system. The stimulation is confined mainly to the medulla and higher centers including the pons, midbrain, and perhaps certain portions of the cerebellum and cerebrum.

(2) The injection of from .0025 to .0050 grams of the salt is sufficient to produce the characteristic action in a medium sized dog, the most conspicuous objective symptom being the production of convulsions. In character, these convulsions correspond to those produced by cyanides and picrotoxin, but, in degree, approximately to those of strychnine.

(3) There is an extreme fall in blood pressure following the first injection of the compound, but a marked and prolonged rise in blood pressure following all subsequent injections. The fall in blood pressure is due to at least two, and probably three factors: (1) Stimulation of the inhibitory vagus center for the heart, (2) sudden dilatation of the vessels of certain visceral organs including the kidney and spleen, and (3) a direct depressant action on the heart. The rise in blood pressure following the second or subsequent injections is due to (1) constriction of the vessels of the kidney, spleen and possibly other organs, (2) stimulation (in the medulla) of sympathetic nerves to the heart, (3) general systemic convulsions in case these are present. But the secondary rise in blood pressure occurs independently of the existence or absence of general convulsions, although these, if present, increase the extent of the rise.

(4) Respiration is stopped by the first injection and greatly increased by subsequent injections. The primary cessation appears to be due to excessive stimulation of the center itself, or, of some higher inhibiting nervous mechanism, probably located in the pons or midbrain. Acceleration and deepening of the respiration, which accompany later injections, are doubtless the result of direct stimulation of the center itself after its hypersusceptibility to the drug has been somewhat decreased by the first injection.

(5) Following the primary injection of lead triethyl acetate, the kidney

and spleen volumes undergo an increase, followed immediately by a decrease, but only the decrease in volume is produced by subsequent injections. The preliminary increase in volume is probably due to stimulation of the vasodilator center in the medulla, while the shrinkage in volume of these organs following the second or subsequent injections is due to a direct stimulation of the vasoconstrictor center. (The preliminary dilatation may, of course, be explained as the result of inhibitory stimulation affecting indirectly the vasoconstrictor center.)

(6) The dyspnea accompanying injections of the drug is not due to spasm of the bronchial muscles, but to a direct action on the respiratory center.

(7) Probably the increased intestinal activity following intravenous administration of the compound is the result of medullary stimulation which thus indirectly increases the activity of the vagus nerves. This may also account for the slow pulse, the increased peristalsis and colic, and the dyspneic, or asthmatic symptoms, of chronic lead poisoning.

(8) The salts of triethyl lead furnish excellent examples of compounds which contain three ethyl groups (which are ordinarily considered to act as depressants on the central nervous system), but which, either owing to the peculiar relations existing in the composition of the molecule as a whole, or else by the specific action of the lead contained in the molecule, act as strong central nervous stimulants, especially in the medulla, pons and midbrain, and perhaps even in the motor areas of the cerebrum, or in the cerebellum.

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STUDIES ON THE RESISTANCE OF THE RED BLOOD CELLS*

I. Resistance of the Red Blood Cells in Health to the Hemolytic Action of Sapotoxin

By Chas. Hugh Neilson, M.D., and Homer Wheelon, M.D., St. Louis, Mo.

THE subject of hemolysis has received much attention both from the experimental and clinical aspect. Early workers confined their attention to the degree of resistance offered by the erythrocytes to anisotonic saline solutions. Since then all types of hemolytic agents have been studied, among which have been animal and plant poisons. Early students sought a physical basis for the processes of hemolysis. Soon, physical laws were found inadequate to explain certain phenomena, and the subject was investigated anew from a chemical standpoint.

In brief the results of the two types of hemolytic action—osmotic tension and hemolysins—are as follows: Rather wide physiologic variations in the isotonic tension of the blood normally occurs. The venous blood usually shows a slightly higher tension than that of the arterial blood. The resistance remains remarkably constant in health but in certain diseases there are found wide variations. Resistance of the red cells to anisotonic salt solutions is raised by the addition of hydrogen, nitrogen, arsenic, carbon dioxide, carbon monoxide, and acids and diminished by traces of alkalies or oxygen. During the course of typhoid fever, pneumonia, erysipelas, and other acute infections the isotonic tension may be increased. The resistance is also increased in leucemia, secondary anemias, pregnancy, lactation, obstructive jaundice and carcinoma. In cases of elevated blood-pressure, hemolytic icterus, fever, chlorosis, pernicious anemia and cyanosis the resistance of the red cells is lowered.

Many hemolytic agents such as the glucosides, specific hemolysins, and animal poisons have been considered from the clinical aspect. Probably the most thoroughly studied hemolytic agent is that of saponin. The saponins markedly reduce the surface tension of water, also they hold insoluble bodies in suspension and have a peculiar affinity for lecithin which they dissolve. Cholesterol, on the other hand, forms an insoluble chemical compound with many of the glucosides. It appears that the presence of a hemolytic agent and lecithin permits destruction of the cells, while cholesterol deprives such poisons of their toxicity by forming inactive cholesterides with them. The injection of saponin results in a terrific destruction of the red blood cells and the appearance of the hemoglobin in the plasma. Such a condition cannot be considered the result of changes in the hemoglobin, but rather to the destruction of the stroma of the corpuscle thereby releasing the hemoglobin. The

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saponins possess a strong solvent action upon the lecithin of the stroma, hence its reduction or removal leads to disintegration of the corpuscles. Such solvent action occurs more readily when the blood cells are removed from their plasma or serum, or suspended in an isotonic saline solution. In such cases the red cells are removed from the protection of cholesterol which normally acts to form inactive compounds with saponin.

Substances other than the saponins when present in the blood stream or about the red cells in excess bring about destruction of the red corpuscles. The action of snake venom has long been known; more recently it has been shown that oleic acid in excess has the same hemolytic action as that of the saponins.

The present paper deals with the action of a specific hemolytic agent, sapotoxin, on the resistance of normal washed and unwashed red blood cells. This work was carried on preparatory to a study of the resistance of the red blood cells in various diseases. The results of our studies on the resistance of the erythrocytes in disease and the relation of red cell resistance to blood chemistry, especially to cholesterol, will appear in later communications. During the course of this study over two thousand experiments were performed.

METHODS

Merck's preparation of sapotoxin was used throughout the series of experiments. The term sapotoxin is employed to designate the more poisonous glucosides, the term saponin being restricted to include less active ones and certain innocuous isomers of sapotoxin which are formed from them by boiling with alkalies. Sapotoxin is a light cream-colored amorphous powder that goes into solution readily; possesses a sweetish, aromatic odor, and froths excessively upon agitation when in solution.

In order to maintain an isotonic solution in which to study the resisting power of the red cells, all sapotoxin dilutions were made up in 0.9 per cent salt solution. A sapotoxin dilution of 1:1,000 was used as a standard or stock solution from which consecutive dilutions of from 1:7,000 to 1:40,000 were made in quantities desired. The solutions were then sterilized and kept on ice while not in use. New solutions were prepared and checked each week.*

The technic for determining the resistance of the erythrocytes to sapotoxin solutions was as follows: A series of from 4 to 6 small, clean test tubes were labeled and placed in a water bath especially prepared for the work, Fig. 1. The temperature about the tubes was maintained between 24½ and 25° C. One cubic centimeter of the desired solutions was delivered into each of the tubes. An extra tube of a 1:13,000 dilution was used to determine the time necessary for complete hemolysis upon standing at a constant temperature. The patient's finger was then prepared and punctured for free flow of blood. By means of a capillary pipette, Fig. 1, 20 c. mm. of blood was drawn and delivered into each of the test tubes and mixed by gentle shaking, the time being noted in each case. Mechanical injury to the cells was re-

*Noguchi has shown that saponins do not deteriorate quickly. However, solutions of sapotoxin are prone to develop growths unless proper precautions are made to keep them sterile.

duced as much as possible as Meltzer and Welch have shown that the erythrocytes have varying resistance to shaking, and that the effect of shaking depends upon the rapidity of vibration.[1] Blood was also obtained for a red cell count and a percentage hemoglobin determination. Having obtained the samples they were removed to the laboratory and each tube allowed to stand exactly five minutes from the time it was delivered into the sapotoxin solution. The tubes were then centrifuged for from 30 to 45 seconds under high

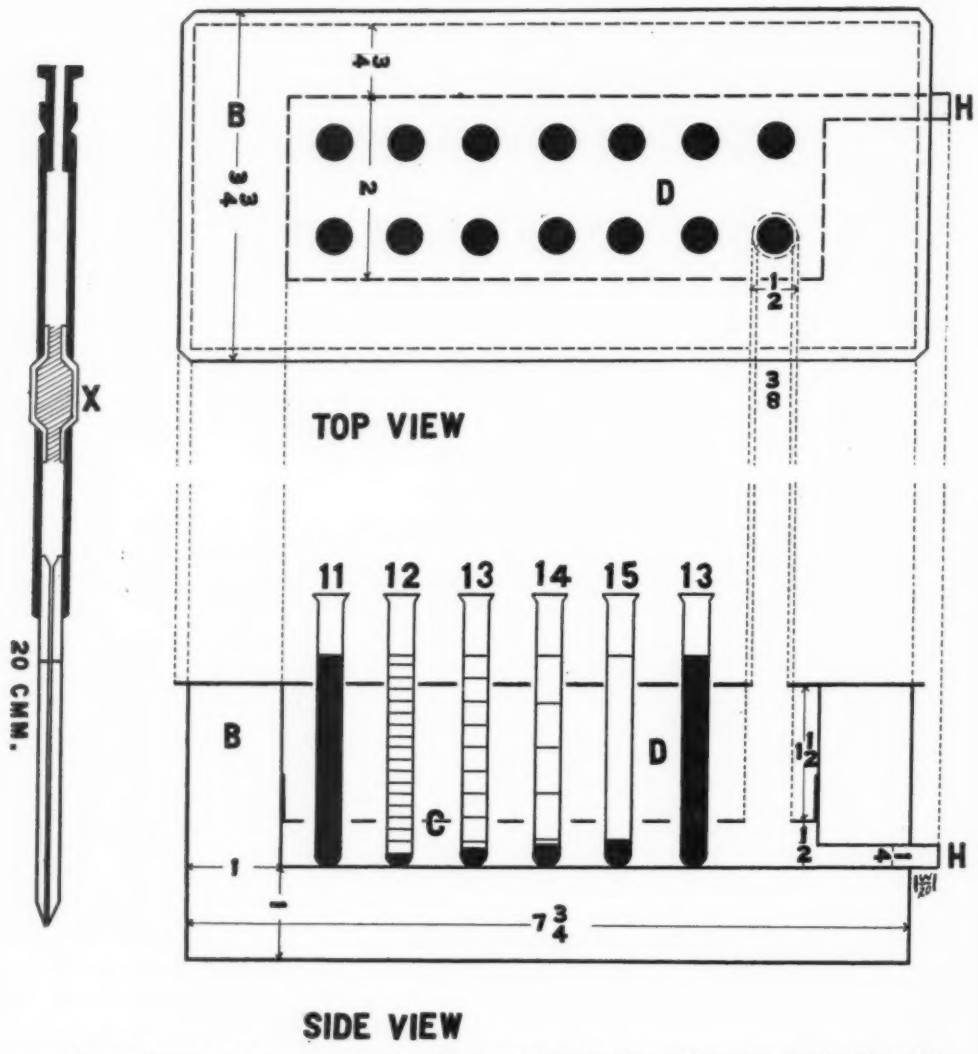

Fig. 1.—Diagram of top and sectional views of test tube container and warmer showing the relation of the double water jacket and test tubes in position. Made of sheet copper to dimensions indicated on drawing. Closed outer jacket *B* has a ¼ inch vent *H*. The inner portion *D* shows holes placed for tubes. A false bottom *C* with perforations corresponding to those of the top but ⅛ inch greater in diameter maintains the tubes in a vertical position. The shadings of the tubes represent various degrees of hemolysis in a given sample of blood at the end of five minutes plus centrifugalization. Hemolysis is shown as complete in tube 1:11,000 strength solution; good in 1:12,000; fair in 1:13,000; trace in 1:14,000; absent in 1:15,000. The second 13 tube, 1:13,000 solution, shows complete hemolysis of red cells on standing at constant temperature. The solid black in the bottom of the tubes indicates the relative amounts of unbroken cells thrown down after centrifugalization.

To the right of the picture is shown a modified Sahli's hemoglobinometer pipette. The portion marked "X" is a piece of glass tubing filled with cotton wool. This simple modification insures evenness of drawing and prevents blood from entering the mouth.

speed. An electrical centrifuge was chosen because of the rapidity with which the unbroken cells could be thrown out of solution. The ordinary water centrifuge is entirely undesirable because of the small number of revolutions possible per minute; its slowness adds to the length of time the red cells are exposed to the action of the hemolytic solution. After centrifugalization the tubes were compared with each other against white light in order to determine the amount of hemolysis secured in each strength of solution. In making readings the following standard was adopted: Hemolysis was considered complete when no deposit of red cells followed precipitation with the centrifuge, the solution possessing a transparent red color. A solution showing a deposit of red cells and possessing a tinge of transparent red was considered as a minimal reaction of the red cells to the solution of sapotoxin to which they had been exposed. Inasmuch as whole blood was used, it was necessary to adopt a minimal red color as the point of minimal hemolysis, because of the straw color normally given by the presence of serum. Degrees of hemolysis between the minimal and maximal were read as good or fair depending upon the degree of hemolysis obtained. In cases where hemolysis was considered fair in one tube and laking failed to take place in the tube next higher in dilution, it was considered that the minimum point lay half way between the two dilutions. For instance, if tube 1:13,000 gave a reading of fair and the next higher dilution 1:14,000 failed to show any degree of laking the minimum point was considered to be a solution whose strength would be 1:13,500.

No difficulty was experienced because of clotting of the whole blood when placed in the testing tubes. The dilution of 20 c. mm. of blood as drawn from the wound in 1 c.c. of sapotoxin solution, and the short period of exposure, 5 minutes, to the hemolytic agent, evidently was enough to allay clotting. However, in certain cases of pronounced jaundice there was a tendency towards clotting, especially in the 1:13,000 solution used to determine complete hemolysis. The same blood was found to give constant results when a constant relationship was maintained between the quantity of blood, solution, time, and temperature.

EXPERIMENTAL RESULTS

The normal red blood cells in 99 cases was found to show an average maximal resistance of 1:13,769. The greatest resistance shown in the series was 1:13,500; the least 1:14,750. The range of variation was, therefore, 1,250 points. In checking new solutions 86 normal determinations were made upon H. W. The average maximum resistance of this series was 1:14,130; the greatest resistance 1:13,500; the least 1:14,500; the greatest variation 1,000 points. The average maximum resistance of the red blood cells for the entire number of normal readings, 185 cases, was 1:13,937. The average time for complete hemolysis to occur in a 1:13,000 sapotoxin solution was 10.7 minutes. The greatest length was found to be 18.5 minutes; the shortest 6.0 minutes (Table I). It is a difficult matter to determine the exact moment of complete laking or the moment of red cell destruction at any period without centrifuging the specimen, hence, the rather wide variations in the time concluded to repre-

sent the end reaction of hemolysis in the 1:13,000 solution. A 1:13,000 sapotoxin solution was chosen rather arbitrarily and early in the work because in most instances this strength solution failed to cause hemolysis in less than 5 to 7 minutes in normal samples of blood. This solution was therefore found convenient because it allowed time for making and completing the other readings. This time method, while inaccurate in itself, acted as a check upon the strength and activity of the other solutions.

For comparative purposes a series of experiments were made upon the resistance of washed red cells to sapotoxin solutions. This was deemed feasible inasmuch as the majority of present work upon blood is done upon washed cells suspended in some form of isotonic solution.

Samples of blood were drawn from the arm veins under aseptic precautions as in obtaining blood for the Wassermann reaction. From 15 to 20 c.c. of blood was usually obtained for the determinations and allowed to clot in the ice chest for 24 hours. It was found that a more compact clot formed and

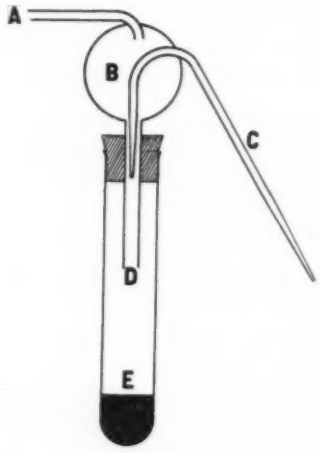

Fig. 2.—Air pump pipette used to withdraw serum from tubes containing coagulated blood. *A*. Connection with air pump. *B*. Large bowl connected with receiving tube *E* through tube *D*. *C*. Receiving tube. Reduction of air pressure in *B* and *E* permits fluid to flow through *C* into test tube *E*.
This pipette, designed by Homer Wheelon, was found to be of special value in the handling of serums and small amounts of solutions.

relatively more serum extruded if the surface of the forming clot was freed from the edges of the tube. After 24 hours' standing the serum was removed from the clot by means of a specially prepared suction chamber pipette, Fig. 2. The clot was then broken up in saline solution and filtered through cotton; the cells greatly diluted with normal saline solution and centrifuged. Washings were continued until the supernatant fluid was free of any coloring matter. Usually 3 or 4 washings were sufficient to remove all serum and laked material. It was found that a 1:1 dilution of the free cells with 0.9 per cent saline solution gave practically a normal red cell count, hence such a dilution ruled out mass action that otherwise might have occurred.

In 12 samples of normal blood the average maximum resistance was found to occur in a 1:37,375 dilution of sapotoxin. In 21 samples of leutic blood the average resistance of the washed red cells was found to be 3,982 points lower

than normal, that is, laking of cells occurred in a solution of 1:41,357 strength. In pregnancy the average resistance of 5 cases was 1:37,100, and in 4 cases of jaundice 36,750. The resistance of 185 cases of normal whole blood (Tables I and II) was 13,936. In 34 samples of syphilitic blood the resistance was

TABLE I

RESISTANCE OF NORMAL WHOLE BLOOD TO SAPOTOXIN SOLUTIONS

Showing the average resistance of the red cells in whole blood to sapotoxin solutions, based upon 99 determinations made on normal individuals and 86 controls made on H. W. Maximum and minimum resistances and maximum differences are shown. (Hem), strength of sapotoxin solution required to give a minimal hemolysis reading 5 minutes. (1:13), time required to bring about complete hemolysis in a sapotoxin dilution of 1:13,000. (Hb), hemoglobin percentage.

	NORMAL SUBJECTS. A.				CONTROLS ON H. W. B.		
	NO.	HEM	1:13	HB	NO.	HEM	HB
MAXIMUM RESISTANCE		13,500	18.5	95		13,500	95
MINIMUM RESISTANCE		14,750	6.0	85		14,500	90
MAXIMUM DIFFERENCE		1,250	12.5	10		1,000	5
AVERAGE	99	13,769	10.7	90.6	86	14,130	93

Average of A + B = 1:13,937 = Normal resistance.

TABLE II

RESISTANCE OF WASHED AND UNWASHED RED CELLS TO SAPOTOXIN SOLUTIONS

Showing the degree of difference in resistance of red blood cells when freed from, and when in contact with, the normal fluids of the whole blood, in normal, jaundiced, syphilitic, tuberculous and pregnant individuals. (No), number of experiments. (Hem), strength of sapotoxin solution required to bring about a minimal laking of red cells within 5 minutes. (1:13), time required, in minutes, to bring about complete hemolysis in a sapotoxin dilution 1:13,000. (Hb), hemoglobin percentage.

	WASHED CORPUSCLES				UNWASHED CORPUSCLES				WASHED CELLS PLUS SERUM	
	NO.	HEM	COUNT	HB	NO.	HEM	1:13	HB	NO.	HEM
Syphilis*	21	41,357	5,086,190	81	34	14,699	11.7	85	4	16,437
Pregnancy	5	37,100	5,280,000	87	35	11,271	16.8	89	3	12,050
Jaundice	4	36,750	5,300,000	85	31	12,685	55.0	79.1	4	12,564
T. B. C.	3	35,000	5,346,000	87	24	12,375	13.1	81.6	3	13,025
Normal	12	37,375	5,100,000	86	185	13,937	10.7	90.6	10	14,050
Average	45	38,900	5,147,775	85	309	13,472	16.0	85.1	24	13,625
Difference	−25,428				+25,428					

*Not on treatment.

14,669; in 35 cases of pregnancy 11,271, and in 31 cases of jaundice 12,685. Therefore, there is an average difference of 25,428 points in the resisting power of the red cells to sapotoxin solutions between the washed and the unwashed cells. Washing decreases the resisting power of the red cells in syphilis by 26,658 points; in pregnancy by 25,829; in jaundice by 24,065; in tuberculosis by 22,625, and in the normal by 23,438 points. Therefore, the serum normally about the red blood cells acts as a direct antihemolytic agent against sapotoxin. This protective action of the serum can also be shown by determining the resistance of washed cells which have been diluted with their own serum. As shown in Table II, the resistance of whole blood in 34 syphilitic

subjects was 1:14,699; in washed corpuscles 1:41,357. A 1:1 dilution of washed cells and serum in 4 cases gave on the average a resistance of 1:16,437, a resistance 1,738 points lower than whole blood and 24,914 points lower than washed cells. Figures are also given for similar determinations upon blood

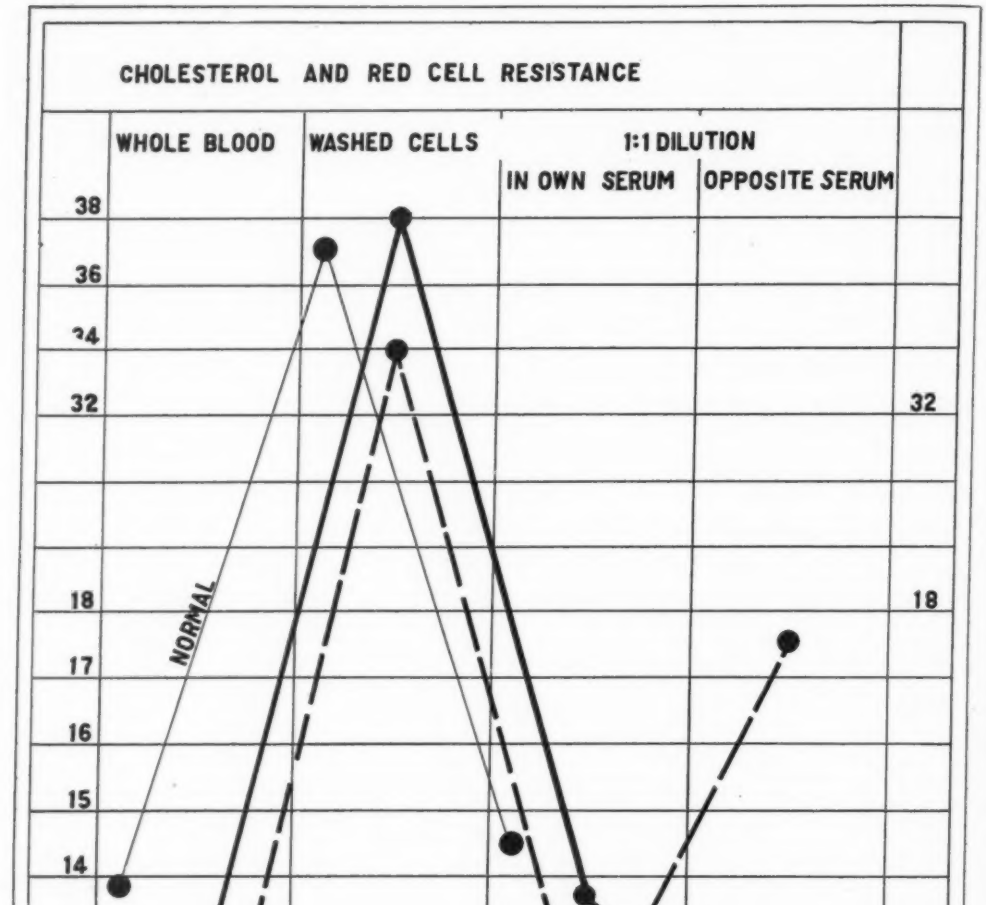

Fig. 3.—Curves showing relation of cholesterol and blood serum to red cell resistance. 66. Case No. 890, jaundiced; cholesterol 166. 292. Case No. 891, obstructive jaundice; cholesterol, 292. Normal. Curve for whole blood, washed cells and washed cells diluted 1:1 with their own serum.
Note the effect of changing the serum relations about the cells of the two cases.

from jaundiced individuals. The relation of the red blood cells to whole blood, isotonic solutions and serum is shown graphically in Fig. 3.

Washed corpuscles are less resistant to the action of bichloride of mercury than unwashed cells. Butler[2] noted that the washing of red blood cells with a 0.9 per cent salt solution caused a distinct lowering of the resistance of the red cells, on the other hand, the washing of cells in a 4 per cent glucose solution did not cause a reduction in cell resistance. Such results lead to the conclusion that the decreased resistance resulting because of washing cells is due to the removal of the protecting material of the serum and to a disturbance of the osmotic conditions. Our results are in agreement with such statements.

Inasmuch as temperature affects chemical action, we chose 25° C. because of the convenience at which such a temperature could be maintained under varying conditions. Noguchi[3] noted that temperature changes greatly affected the reaction time of hemolytic agents; an increase in temperature decreases the laking time, while a reduction in temperature increases the time required to bring about the same degree of hemolysis. An experiment performed to demonstrate the relation of temperature changes upon the power of sapotoxin to bring about a minimal hemolysis gave the following results: Resistance readings at temperatures 15, 20, 25, 30, 35, 40 and 45 degrees centegrade were 1:10,000—1:13,250—1:14,100—1:18,000—1:21,000—1:24,000—and 1:26,000, respectively. Hence, an increased temperature decreases the strength of sapotoxin required to lake the same mass of red blood cells (20 c. mm.) in a given time. The maximal resistance as determined in a 1:13,000 sapotoxin solution for temperatures from 15° to 45° C. were 18, 14, 8, 5, 3, 2, 1.7, and 1 minutes, respectively. Hence, an increased temperature determines a more rapid rate of destruction of the red cells exposed to a given strength of solution.

SUMMARY AND CONCLUSIONS

I. A rapid method is described for the determination of the degree of resistance of the red blood cells to a specific hemolytic agent—sapotoxin. The average maximal resistance of the corpuscles in the whole blood of 99 individuals chosen as normals was a 1:13,769 strength sapotoxin solution. Eighty-six determinations on H. W. over the course of the experiments averaged a 1:14,130 strength solution. The average of all normal readings—185—was a 1:13,937 solution. The average length of time for complete hemolysis to occur in a 1:13,000 solution at a constant temperature—25° C.—was 10.7 minutes. The average hemoglobin as determined by the Tallquist hemoglobinometer was 91 per cent for all cases. Washed corpuscles from 12 normal cases suspended in isotonic salt solution were found to show a minimal degree of hemolysis in a 1:37,375 sapotoxin solution. Findings in leutic, pregnant, and jaundiced cases are also given. Washed corpuscles diluted 1:1 with normal saline solution gave practically a normal count, hence mass action was ruled out because of this dilution. Also, washed corpuscles diluted 1:1 with their own serum demonstrate practically the same resistance against sapotoxin as cells present in whole blood. The red blood cells normally show a remarkable degree of constancy in their resistance to a specific hemolytic agent.

II. Therefore, it may be concluded that the presence of the blood fluid about the red cells acts in such a manner as to resist the hemolytic action of sapotoxin.

REFERENCES

[1]Meltzer and Welch: Jour. Physiol., 1884, v, 225.
[2]Butler: Quart. Jour. Med., 1913, vi, 145.
[3]Noguchi: Jour. Exper. Med., 1906, viii, 337.

LABORATORY METHODS

A TEST FOR EARLY RENAL INSUFFICIENCY*

PRELIMINARY PAPER

BY THOMAS BYRD MAGATH, M.D., ROCHESTER, MINN.

FOR the past two decades many attempts have been made to study the renal function of patients who have demonstrable primary lesions of the kidneys, or lesions that affect the kidneys secondarily. As the result of all the methods known, many have claimed that the most generally valuable individual test is the estimate of a single sample of blood urea. Many investigators, however, have tried to find some test that would demonstrate an earlier renal involvement than that which can be shown by an already elevated blood urea. Thus the diagnostic determination of blood uric acid has been developed and it has been shown quite clearly that there may be an elevated blood uric acid in renal involvement long before the blood urea is above normal, because the kidneys excrete uric acid with the greatest difficulty of any known nonprotein substance. The determination of uric acid is of more value than urea in the early diagnosis of nephritis.

In cases in which the blood uric acid is marginal; that is, between 2.5 mg. and 3.5 mg. for each 100 c.c. the question often is raised of whether there is any significance in the results. It was thought possible to answer this question and at the same time demonstrate earlier lesions than could be shown by a single blood uric acid determination, if the patient were tested for his ability to excrete uric acid. Denis showed some years ago that patients with nephritis exhibit an accumulative effect of uric acid in the blood if fed on high purin diets, while normal persons have no change in the blood uric acid under these conditions. Upham and Higley pointed out the necessity of having patients on a constant purin intake in order to study and compare blood uric acid determinations. It seemed logical, therefore, if a patient were fed uric acid and there was a slight lesion of the kidneys too small even to give a very marked rise of blood uric acid with a normal diet, that it might be possible to demonstrate an early inability of the kidneys to excrete uric acid and thus determine a possible prenephritic condition. The test was carried out as follows:

The patient was put on a purin-free diet (milk) for three days and the amount of uric acid excreted in the urine was determined for the third twenty-four hours. The method of Folin and Wu was used throughout this work. Three-fourths of the weight of the patient was ascertained and calculated for the weight of body fluids. Enough uric acid was given by mouth to cause a rise in the blood uric acid of about 2.5 mg. for each 100 c.c. if the patient failed

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to excrete any of it. This usually meant the administration of about 2 gm. of pure uric acid. A determination was then made of the uric acid in the blood. The uric acid excreted during the next twenty-four hours was determined; at the end of this time a second blood uric acid determination was made and compared with that made at the time the drug was administered. It is evident, therefore, that there is a check on any uric acid that might be lost by changes in the rate of absorption in the gastrointestinal tract.

Three patients were tested in the manner described. None of the three presented blood or urinary findings by the usual methods sufficient to warrant the diagnosis of renal involvement, yet all three had a decided hypertension with practically no other physical findings. In one case the patient excreted, prior to the administration of uric acid, 0.4 gm. in twenty-four hours. At the time he was given uric acid, the blood contained 2.5 mg. for each 100 c.c. At the end of the next twenty-four hours the patient had excreted 0.34 gm. of uric acid, and the blood determination showed a concentration of 5.8 mg. for each 100 c.c. Since the other two cases ran parallel, only one will be cited. The patient excreted 0.5 gm. of uric acid in twenty-four hours, prior to the administration of the drug and showed a blood uric acid of 2.8 mg. for each 100 c.c. At the end of twenty-four hours the blood uric acid concentration had not changed and about 70 per cent of the uric acid was recovered in the urine. The first case was interpreted as a prenephritic condition. The other two must, for the present, be considered as essential hypertensions without renal involvement.

The uric acid used for these tests was prepared from human urine by the usual method. Several other cases were tested as here outlined; a commercial product of uric acid was used. There was neither rise in the uric acid of the blood nor of the urine, indicating that the failure might be due to the fact that the uric acid was probably not absorbed in the gastrointestinal tract, at least not as uric acid. This result is in keeping with observations made by investigators in the past.

Caffeine citrate was used in other cases, and here I was able to obtain calculated rises in the blood or urine according to whether the individual was normal or nephritic. From this it would seem that caffeine is excreted as uric acid or piles up as uric acid in the blood in cases of nephritis or else some other substance is formed that gives a blue color by the Folin and Wu method. The dose used was about 1 gm. of caffeine citrate in capsules at three one-hour intervals just prior to the ingestion of a meal.

The method by which caffeine is excreted is still a question of controversy. Sollman asserts that it is completely and readily absorbed, that very little is excreted as such, and that from 10 to 40 per cent of the substance loses its methyl groups and is excreted as dimethylxanthin or monomethylxanthin; up to 80 per cent is excreted as urea, practically none as uric acid. Cushney asserts that the uric acid of the urine is not increased by the ingestion of caffeine. On the other hand Greene believes that caffeine is demethylated in the body and forms xanthin which is excreted in the manner in which any animal xanthin is excreted, namely as uric acid. Schittenhelm found that feeding caffeine increases the amount of uric acid in the urine. Taylor con-

TABLE I

NO LESIONS OF THE KIDNEY

CASE	DIAGNOSIS OF NEPHRITIS (CONNER)	BLOOD URIC ACID 1	BLOOD URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	URINE URIC ACID 1	URINE URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	REMARKS
325661	Syphilis	3.4	3.4	±0.0	0.47	0.69	+0.22	
343023	Neurosis, migraine, syphilis	1.3	3.1	+1.8	0.41	0.40	−0.01	Treatment for syphilis
342157	Pulmonary tuberculosis	3.2	2.4	−0.8	0.25	0.86	+0.61	
342901	Bronchial asthma, prolapse of ovaries	2.1	1.9	−0.2	0.31	0.37	+0.06	0.4 gm. caffeine*
341589	Artificial vagina	2.0	2.8	+0.8	0.15	0.27	+0.12	
340455	Epilepsy, central nervous system syphilis	2.5	2.9	+0.4	0.29	0.38	+0.09	Treatment for syphilis
342960	Leukorrhea	1.8	2.0	+0.2	0.38	0.62	+0.24	
342204	Metrorrhagia, neurosis	2.3	1.9	−0.4	0.19	0.81	+0.62	
341076	Chronic sinusitis	2.4	1.7	−0.7	0.68	0.89	+0.21	
316534	Central nervous system syphilis, cystitis	2.3	3.3	+1.0	0.76	0.62	−0.14	Treatment for syphilis
340982	Diarrhea, pancreatitis	2.0	2.2	+0.2	0.40	0.42	+0.02	

*Full dose 0.8 gm. caffeine.

TABLE II

LESIONS OF THE KIDNEY (?)

CASE	RENAL DIAGNOSIS	CLINICAL DIAGNOSIS (OTHER THAN RENAL)	BLOOD URIC ACID 1	BLOOD URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	URINE URIC ACID 1	URINE URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	REMARKS
341014	Hypertrophy of prostate	Probably slight nephritis	3.1	2.3	−0.8	0.60	0.77	+0.17	
343173	Exophthalmic goiter	Probably mild chronic nephritis with arteriosclerosis	3.8	3.2	−0.6	0.73	0.51	−0.22	Twelve-hour specimen urine
342739	Pericarditis	Possibly mild pyelonephritis	1.7	1.9	+0.2	0.30	0.25	−0.05	0.4 gm. caffeine*
342897	Bronchial asthma	Probably mild nephritis	3.0	2.8	−0.2	0.58	0.58	±0.00	Eighteen-hour specimen urine
340069	Myocarditis	Possibly nephritis	3.0	3.0	±0.0	0.56	0.54	−0.02	
341588	Chronic constipation; psychoneurosis	Possibly mild nephritis	1.4	2.5	+1.1	0.63	0.74	+0.11	
224717	Dental infection, nervousness	No lesion of the kidney ?	1.4	1.7	+0.3	0.28	0.50	+0.22	
342605	Anemia, gastritis	Probably mild nephritis	1.4	1.8	+0.4	0.28	0.32	+0.04	
340597	Hernia	No lesion of the kidney ?	2.4	3.1	+0.7	0.19	0.46	+0.27	
338792	Hypertension	Probably some chronic nephritis	2.6	3.3	+0.7	0.55	0.46	−0.09	
433090	Achylia gastrica, pyorrhea	Possibly mild chronic nephritis	3.6	3.8	+0.2	0.67	0.42	−0.25	

*Full dose 0.8 gm. caffeine.

TABLE III

DEFINITE LESION OF THE KIDNEY (CLINICALLY)

CASE	GENERAL DIAGNOSIS (CONNER)	BLOOD URIC ACID 1	BLOOD URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	URINE URIC ACID 1	URINE URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	REMARKS
338961	Chronic nephritis, arteriosclerosis	2.0	2.8	+0.8	0.42	0.29	−0.13	0.4 gm. caffeine,* eighteen hour specimen of urine
342561	Left pyelonephritis with chronic nephritis	3.8	3.3	−0.5	0.70	0.99	+0.29	
341707	Hydronephrosis with stones, nephritis	1.9	2.5	+0.6	0.26	0.39	+0.13	
340546	Infected hydronephrosis	2.4	3.1	+0.7	0.13	0.42	+0.19	
341689	Mild nephritis, arteriosclerosis, hypertension	3.0	3.5	+0.5	0.83	1.00	+0.17	
339900	Mild nephritis	2.2	3.0	+0.8	0.26	0.28	+0.02	
341621	Mild chronic nephritis, hypertension, arteriosclerosis	2.1	2.8	+0.7	0.90	0.81	−0.09	
342111	Chronic nephritis, hypertension	3.4	3.8	+0.4	0.43	0.40	−0.03	
342499	Nephritis, arteriosclerosis, hypertension	1.6	2.7	+1.1	0.77	0.54	−0.23	
343063	Chronic nephritis	3.7	3.7	±0.0	0.39	0.53	+0.14	0.4 gm. caffeine*
338719	Mild nephritis	3.2	2.8	−0.4	0.44	0.48	+0.04	Gout ?
341339	Chronic nephritis, hypertension	2.8	2.8	±0.0	0.37	0.65	+0.18	0.4 gm. caffeine*
340860	Mild nephritis	2.4	2.2	−0.2	0.37	0.98	+0.61	
341816	Chronic nephritis	2.5	2.8	+0.3	0.70	0.42	−0.28	Eighteen hour specimen urine
342234	Right kidney infected	2.0	2.4	+0.4	0.52	0.68	+0.16	
343473	Mild nephritis	3.2	3.9	+0.7	0.62	0.36	−0.26	Eighteen hour specimen urine

*Full dose 0.8 gm. caffeine.

cluded that coffee added to the diet increases the output of uric acid quite markedly, and explained it on the basis of the caffeine in the coffee. Benedict repeated, to some extent, the work of Taylor and concluded that caffeine at least when ingested as coffee increased the output of uric acid in the urine.

This caffeine test has been used in a number of cases which were studied clinically and diagnosed in relation to the condition of the kidney. The cases were divided into three groups, those without lesions of the kidney, those with questionable lesions from a clinical study, and those with definite lesions clinically. The results are shown in Tables I, II, and III. It may be seen that in general the caffeine test checked very nicely the clinical findings. Three patients (Table I) are marked exceptions to this statement. All were on intensive antisyphilitic treatment and it is possible that they had very slight kidney lesions, which must be quite common with intensive treatment with mercury and arsenic, but cannot be detected clinically. One other exception to the clinical findings is seen in Table III. A man with a left pyelonephritis had a free output of uric acid, which may be explained on the basis of a competent right kidney. A case diagnosed mild nephritis with some question of gout showed practically no increase in the output of uric acid, but rather a little diminution in the second blood specimen taken. This finding is interesting in view of the fact that uric acid is believed by some observers to be retained in the tissues in cases of gout.

Recently McLean and Wesselow have used a urea concentration test showing that earlier renal involvements may be demonstrated than by a simple blood urea estimation. The test consists, briefly, in feeding the patient 15 gm. of urea and determining the amount excreted in the urine in two hours' time. It remains to be seen whether this test furnishes more information than a single uric acid determination, and certainly from a theoretical standpoint it could not show as early a renal involvement as the proposed uric acid concentration test outlined in this paper. These tests were performed many months before the publication of the work of McLean and Wesselow, in an attempt to demonstrate the rôle of renal insufficiency in essential or idiopathic hypertension. The pressure of other investigations, however, has made it impossible to carry on the work at the present time and this note is published in the hope that others interested in such cases will give the test a trial and will perhaps obtain a series that may be compared with other renal functional tests.

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EDITORIALS

Syphilology and Clinical Synthesis

OSLER said, once upon a time, that Jonathan Hutchinson was the greatest generalized specialist or specialized generalist in medicine because he knew syphilis in all of its ramifications. He also said, by way of corollary, that if a man knows syphilis he knows medicine.

Syphilis is one of the few diseases in which interest never fails and about which a special literature grows at a rather even rate. The curve of interest in the Great Pox does not seem to have a form resembling that of the old Baltimore and Ohio Railroad—or the Erie; it is not a malarious curve. Other infectious diseases have their ups and downs. Syphilis follows a straight line. The general and uniform interest in the disease is reflected in the fact that the Wassermann reaction has become a matter of routine in clinical studies.

But while the laboratory test is an aid to the physician, its introduction and widespread use has perhaps overemphasized the mere laboratory side of diagnosis to the extent that many men trust rather to it than to their observational skill in studying cases of syphilis or of cases in which syphilis is a possible factor. There is a tendency to forget that the Wassermann reaction like any other laboratory test is a thing which adds something to a clinical

history, and that it is the entire clinical history upon which a diagnosis rests. Not only diagnosis but prognosis is prone to suffer from the general clinical disregard which follows too complete dependence on the laboratory. So as Stokes and Busmann say, reversal of the Wassermann reaction while desirable should not be the primary aim of the therapy. Symptomatic response with arrest of the process, and the giving of as much treatment as tolerance permits are the chief considerations.

Stokes has evidently been through the so-called laboratory stage for he has emerged from that "monastic phase in response to a new appreciation of the enduring worth and ultimacy of clinical research." The laboratory has become his assistant, rather than his master. The critical problem of the next half century of syphilology, he says, is not so much the extension of laboratory knowledge now so highly developed, as the application of the knowledge already existent, to the clinical problems of the disease. No reasonable man would seek to minimize the worth of the laboratory contribution. But a decade of transformations has been superimposed on the clinical syphilology of Fournier, Hutchinson and Morrow as two fluids of different densities may be superimposed the one upon the other in a test tube. What is needed now is a mixture, a solution. The day of the *pousse café* is past.

Stokes' experience has taught him that syphilis is no longer a problem of the dermatosyphilographer alone, and that the day of the syphilographer who can combine in his sole personality both the technical attainments and the wealth of general and detail knowledge that make him master of the disease, is past. He verifies Osler.

He has made a list of the examinations on which, at one time or another, his department relies for a diagnosis or information on the status of a syphilitic infection, and a glance at this list will show how illusory is the hope that syphilis will ever yield to a single diagnostic key. The rapid modification of clinical knowledge under the pressure of new technical diagnostic and therapeutic methods has induced a peculiar state of disorganization in the syphilologic realm. True syphilology is, for the time being, lost in the mazes of an analytic era and the syphilologic workshop is crowded with personalities each so intent on the part which he contributes that he can give no thought to the whole. The syphilologist of the future is as yet in a phase of mental dissociation suggesting the unfortunate condition of the Miss Beauchamp of Morton Prince's study. Synthetic studies are needed.

In the present state of affairs, with the trend of medicine more and more to specialism, groups have grown up and the group system enlarges itself just because synthetic studies are so obviously needed. But as yet the system is very imperfect except in a few instances. When the system is perfected every doubtful case, whether of syphilis or what-not, will be the subject not not only of sporadic clinical notes from each division of the group, but of a round table conference at which with the clinicians the laboratory man will have a seat. Then the previous analyses can be studied and synthetically estimated. In such conferences each specialist can get and will get a bird's-eye view of a subject matter and of methods that are largely foreign to him, and a clinical perspective will gradually develop. There will be less flatness

in office and laboratory work and clinical stereoscopy will be developed. The laboratory will no longer be a monastery from which edicts are issued, but will become a cooperative part of the group from which will come opinions and suggestions which will be discussed and evaluated by all the members.

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—*P. G. W.*

The Spread of Bacterial Infection

TOPLEY[1] has made an interesting experimental study of an epidemic among mice. A certain number of these animals were fed cultures of B. Gaertner and from time to time healthy mice were added to the infected community. Effort was made to be sure that the additions were in health. They were taken from batches of from six to eighteen which had been kept in cages for two or more weeks and in which there was no evidence, either by disease or death, of the presence of an infection. These healthy mice were added to the infected cage from time to time. The experiment continued through three hundred eighty-eight days, during which time seven hundred eighty-two mice were added and seven hundred twenty-eight deaths occurred. It was found that the percentage of deaths increased when the fresh animals were added during the ascent of a mortality wave. When fresh animals were added while the mortality was on the increase not only was death more frequent among the additions, but also increased among those already in the cage and which had survived other epidemic waves. The author says: "In the present study it has been shown that if normal mice are added from day to day to an infected population they will all eventually succumb, provided that further normal mice are added at a certain rate. The period of survival of any given batch of mice will vary according to the time at which they are introduced to the cage. If their entrance coincides with the early part of the rise of an epidemic wave, as judged by a mortality curve, their survival will be short. If they are introduced during the latter part of the decline of such a wave they will live, on the average, much longer, longer indeed than mice subsequently added to the cage."

By frequent addition of susceptible material to an infected community, an epidemic may apparently be prolonged indefinitely. If this be applicable to epidemics among human beings, it is quite evident that quarantining an infected community is desirable and beneficial not only for protecting those outside such community, but with the object of limiting the deaths within the community itself. We are of the opinion that we observed something like this in our cantonments during the late war. It was certainly evident that with each additional accession of fresh material to an infected camp the number of cases and the number of deaths increased and the increase involved not only those who came into the camp, but those who were originally in it.

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[1]Jour. Hygiene, 1921, xix, 350.

Topley states his conclusions as follows: "(1) If susceptible mice be continuously added to an infected population the spread of infection will continue over a long period of time. There is no evidence that this period has a limit. (2) When susceptible mice are added continuously and at a constant rate to an infected population, the spread of infection, as judged by a mortality curve, is propagated in regular recurring waves. These waves are most easily observed by noting the fluctuations in the total cage-population. It seems probable that the period of these fluctuations will be found to depend on the rate of addition of susceptible individuals, but this point has still to be determined. (3) The actual deaths may occur in large groups, with intervals during which deaths are few and far between, or they may fall in a succession of smaller groups, increasing and diminishing in size to form the larger waves. In all cases there is this tendency for the occurrence of such small groups of deaths with definite maximal points. There would seem to be two fluctuating processes, the one superimposed upon the other. (4) The average survival-time of mice added to the cage, and their chance of ultimate survival if no more susceptible mice are introduced, vary according to the phase at which they are added. If they gain entrance to the cage during the rise of a wave they are unlikely to live for long. If they are introduced during the fall of a wave their chances of survival are greatly increased, and they will usually outlive mice which are added at a later date but at a time before the commencement of the next wave. (5) The rate of extinction of a population, among which infection is actively spreading, will be far less rapid if they are kept isolated, than if further susceptible individuals continuously gain access to them. A proportion of the infected population, which would have survived indefinitely under the former circumstances, will die under the latter. (6) The ultimate survivors among such a population have not escaped infection, but have successfully resisted it. A considerable proportion of them are harboring the causative parasite in their tissues."

We are tempted to theorize after reading the above mentioned report, but have concluded that theory had better be omitted until more experimental work along this line has been done. Incidentally, however, we cannot help recalling the fact that we would not have known of yellow fever in Cuba had it not been for the susceptible accessions that were constantly coming into the Island. —*V. C. V.*